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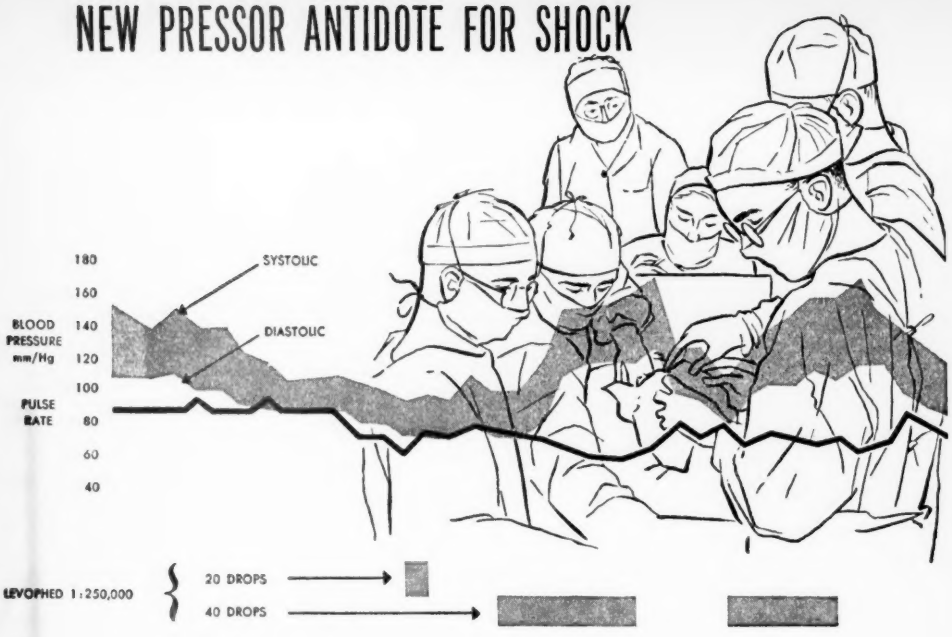

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SOME ASPECTS OF THE TRANSFER OF OXYGEN ACROSS THE SYNDESMOCHORIAL PLACENTA OF THE SHEEP*

DONALD H. BARRON

Since the classical studies of Grosser[14] the classification of mammalian placental types has been based upon the number of tissue layers separating the maternal vascular system from the fetal vessels and across which the diffusable materials essential to intrauterine development must move from the maternal to the fetal plasma. Grosser's[1] classification is based on the concepts: (i) that in phylogeny as in ontogeny the simplest or primary stage in placentation is one in which the barrier consists of the following intervening structures—the maternal vascular endothelium, the maternal connective tissue, the maternal epithelium, the cavity of the uterus, the chorionic epithelium, chorionic connective tissue and endothelium; and (ii) that the phylogenetic development of the placenta involved the destruction of the tissue layers separating the maternal blood from the uterine cavity one after another as in implantation during ontogeny.

First, the maternal epithelium breaks down either before the application of the chorionic epithelium of the embryo to the uterine wall or after the obliteration of the uterine cavity at the site of implantation, i.e., after the apposition or fusion of the fetal and maternal epithelial layers. Next, the maternal connective tissue is absorbed—the endothelium alone remains and finally this, too, is destroyed and the maternal blood bathes the fetal epithelium. From his studies Grosser concluded that only the maternal tissue layers were destroyed in the ontogenetic development and phylogenetic evolution of the placenta and accordingly he assumed the highest type or final stage in development to be that in which the fetal epithelium was in direct contact with the maternal blood; but the more recent observations of Mossman[19] demonstrate that there is yet another stage—one in which the fetal epithelium and connective tissue disintegrate. The maternal and fetal bloods are in this stage separated by but a single layer of cells, the fetal vascular endothelium.

The basic phylogenetic pattern of the placenta according to Grosser's scheme—consisting of six layers, three maternal and three fetal, separated by a potential space representing the obliterated uterine cavity—is found in the pig and in the horse. In the ruminants—the sheep, the goat, and the deer—the maternal epithelium is lost to a greater or lesser degree, and in some areas the connective tissue is destroyed. The slit-like cavity of the uter-

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us disappears through the adhesion of the chorion and maternal epithelium or, after its destruction, the maternal connective tissue. In the carnivores only the maternal endothelium remains to be invested by the fetal chorion. The final stages are illustrated in the primates and rodents; in all of these forms the maternal tissue disappears and the fetal tissues dip into the maternal blood; in the rabbit and guinea pig the fetal epithelium and connective tissue disappear during the final stages of gestation and the maternal blood is in contact with the fetal vascular endothelium.

The nomenclature Grosser suggested for the classification of placentae according to his scheme is as follows: Placentae in which the chorionic epithelium is applied to the uterine epithelium are called epithelio-chorial; those with intact maternal connective tissue, syndesmo-chorial; types in which chorionic epithelium is applied to maternal vascular endothelium are designated as endothelio-chorial, and forms in which fetal chorion is bathed by maternal blood as haemo-chorial. The first part of the combined word gives the last maternal layer through which the diffusable elements pass en route from mother to fetus. Mossman followed this same scheme and classed the final stage of the development of the rabbit and guinea pig placentae as haemo-endothelial. One must recognize that these types or stages are not sharply defined and that a given classification implies only that the layers in apposition over the major part of the surface of contact between fetal and maternal tissues are of the type indicated.

Grosser's scheme for classification is an especially attractive one, based as it is on a consideration of the dynamics of the transfer of materials from the mother to the fetus and vice versa; it has served to focus attention on the rôle the different tissue layers forming the placenta may play in the active transfer or transportation of materials between the two blood streams and on the nature of the barrier each type might provide to limit the transfer of materials that move across the placenta by simple diffusion. Moreover, it leads to a consideration of the manner in which these differences in placental structure influence the mechanisms through which the mother provides the essentials for the fetus and the circumstances affecting the access of the fetus to them.

A variety of aspects of the comparative physiology of the transfer of materials between mother and fetus have been under investigation; the influence of the placental structure on the diffusion of sodium has been analyzed in a series of classical experiments by Flexner[8-11] and his colleagues Pohl[20, 21] and Gellhorn.[12, 13] Their comparative studies demonstrate quite clearly that the phylogenetic and ontogenetic changes in the structure of the placental barrier are associated with alterations in permeability; the placentae that appear on morphological grounds to be less permeable are indeed less so. Other studies indicate that there are upper limits to the size of the molecule that can move from mother to fetus which are associated with the thickness of the barrier (Brandstrup[6]); in general, the greater the number

of tissue layers separating maternal and fetal blood, the smaller the molecules that gain passage. But there is another aspect of the problem in which I have been interested, i.e., how does the nature of the placenta affect the transfer of oxygen from the mother to the fetus? Specifically, how does it affect the O_2 pressure gradient across the placenta and what are the demands put upon the mother to maintain that gradient? As the first step toward the answers to these questions the following studies on the O_2 transfer across the syndesmochorial placenta of the sheep were undertaken; similar studies are now in progress on the O_2 exchange across the haemoendothelial placenta of the rabbit.

As the first stage in this study I compared the oxygen dissociation curves of normal barren ewes with those fetal and maternal bloods at several stages in gestation; with these curves, if the percentage saturation of a blood sample taken from the fetal or maternal vessel was known, the oxygen pressure in that sample could be estimated. In this way the pressures were estimated in the uterine artery, the uterine vein, the umbilical artery, and umbilical vein. These pressures were used in turn to estimate the gradient between the fetal and maternal bloods at the two ends of the parallel capillary cords in the cotyledon. Finally, as the third aspect of this study, the coefficient of utilization of the uterus and its contents was estimated by subtracting the oxygen content of the uterine vein from that of the uterine artery and dividing the difference by the content of the artery; the quotient multiplied by one hundred gives the coefficient of utilization as defined by Krogh.

Materials and Methods

For these studies thirty-three ewes were available; they were good grade sheep, some Dorsets, some Cheviots, and others were Shropshires. They were kept and settled on known dates at a farm outside of New Haven* where they had the run of a good pasture and in the winter months alfalfa hay ad libitum with a daily ration of grain. Of these ewes six were barren; samples of their blood were used for the preparation of normal dissociation curves. The remaining twenty-seven were sacrificed on selected dates following insemination to provide data at fairly regular intervals throughout the entire gestation period. Of these twenty-seven ewes, nineteen bore single lambs and eight twins. The ages of the fetuses on the dates of sacrifice, together with data obtained from the studies of their blood, are indicated in Table 1.

The ewes selected for study were brought into the laboratory four or five days before the experiment and kept off feed about forty-eight hours. The blood, 50-60 cc., for the dissociation curves of the ewes whether pregnant or barren was drawn from the external jugular vein; the ewes were unanesthetized and usually standing. After the blood was drawn the pregnant ewes were given 1.5-2 cc. of Spinocain subdurally and placed on their backs; the abdomen was then opened by a midline incision that extended from a point just below the xiphisternum to one just above the symphysis pubis. The uterus was gently maneuvered into a favorable position within the abdomen with as little exposure or handling as possible.

* I am indebted to Dr. and Mrs. Morton Loeb of Bethany, Connecticut, who cared for the sheep as a part of their flock. Without their interest and co-operation the experiments could not have been carried out.

One of the small veins draining a cotyledon of the central portion of the horn of the uterus occupied by the fetus was selected and without dissection or direct handling a 2-5 cc. sample of blood was drawn from it under oil into a syringe containing a mixture of heparin and fluoride in the dead space to prevent clotting and self-reduction

TABLE 1

A LIST OF THE EWES USED IN THE EXPERIMENTS DESCRIBED AND THE STAGES IN GESTATION AT WHICH THEY WERE SACRIFICED (FULL TERM 145-150 DAYS) TOGETHER WITH AN INDEX OF THE DATA OBTAINED WITH THE SAMPLES OF THE MATERNAL AND FETAL BLOODS

Ewes with singlets	*Fetal age Days*	*Oxygen dissociation curves*		*Coefficient O_2 utilization*
		Maternal	*Fetal*	
59	50	x	—	x
2	56	x	x	x
54	62	x	x	x
58-51	76		x	x
9C	80	x	x	x
23	81	x	x	x
32	85	x	x	x
5R-51	92		x	
30	96	x	x	x
26	108	x	x	x
50	111	x	x	x
55	119	x	x	—
19C	121	x	x	x
24	122	x	x	x
68	126	x	x	x
3	136	x	x	x
11	136	x	x	x
53	139	x	x	x
60	140	x	x	x
Ewes with twins				
9	50	x	—	x
9 gr.-51	65		x	
8	81	x	x	x
52	116	x	x	x
5	120	x	x	x
58	121	x	x	x
70	126	x	x	x
19	137	x	x	x

of the blood. The uterus was then opened with a single incision running at right angles to the long axis of the horn and the fetus gently but quickly drawn from the uterus into the abdominal cavity. Samples of blood were similarly drawn from the umbilical vein, the umbilical artery, and the uterine artery as rapidly as possible and with the minimum of disturbance to the fetus and the uterus. After the needles had been removed and the syringes capped they were put into beakers containing ice and water. Finally, a larger quantity, depending upon the age of the fetus and the amount

of blood available, was drawn from the umbilical vein for the preparation of the fetal dissociation curve. The number of points determined was in those cases younger than eighty days determined by the blood available.

The oxygen content and capacity of the smaller samples were determined by the manometric method of Van Slyke; these two values were used to calculate the percentage saturation. In the preparation of the oxygen dissociation curves, determinations were usually made of the percentage saturation of the haemoglobin of the blood at four different oxygen pressures, *ca.* 20, 40, 60, and 80 mm. Hg, except in those cases in which the fetus was younger than eighty days and the volume of blood available was not sufficient for the determination of four points; on No. 54 (62 days) only one point was obtained and but two for each individual on Nos. 59 and 2 (50 and 56 days respectively). Five cc.'s of blood were introduced into tonometers of about 270 cc. in capacity and filled with gas mixtures containing differing amounts of oxygen; sufficient CO_2 was added to each tonometer so that the pressure would be 40± 2 mm. Hg on equilibration. The tonometers were rotated in a water bath held at 38° C. About five minutes after they were put into the bath the stopcock of each one was opened just beneath the surface of the water in the bath, and the gas was allowed to escape so that the total pressure at equilibrium was that of the atmosphere. Thereafter the rotation of the tonometer in the bath was continued for another fifteen minutes; equilibrium was established in that time.

Samples of blood were withdrawn from the tonometers into well-oiled syringes by means of a needle thrust through the rubber stopper that closed one end of the tonometer. After the needle was removed the syringes were capped and the bloods stored in them until they were transferred to the pipettes for the introduction of measured volumes into the manometric apparatus of Van Slyke for the determination of oxygen content. All the determinations of the oxygen content were done in duplicate on 0.5 cc. samples except those on the bloods of fetuses younger than 80 days; the quantity of blood available from these was so small that the analyses were done in duplicate on 0.2 cc. samples.

The gases in the tonometers were analyzed by means of the Henderson-Haldane apparatus, and the oxygen and carbon dioxide pressures were calculated from the barometric pressure after correction for the water vapor pressure at 38° C. With these results and those of the percentage saturation of the bloods at different oxygen pressures, dissociation curves were constructed for the fetal and maternal bloods. The oxygen pressures in the small samples drawn from the umbilical and uterine vessels were estimated by referring their percentage saturation to the appropriate curves.

These curves were used for the estimation of the oxygen pressure in the venous samples as well as the arterial, since data at hand (unpublished results) indicate that the increase in oxygen pressure required to hold the saturation of the maternal blood constant at 70 per cent—a saturation characteristic of blood from the uterine veins—as the CO_2 pressure is increased from 40 to 50 mm. Hg varies in my experiments between 0.3 and 0.4 mm. per mm. increase in CO_2 tension. As the CO_2 pressure in the maternal blood increases 3-5 mm. as it passes through the placenta, the oxygen pressure required to bring about the venous level of saturation at the elevated CO_2 pressure would not exceed that required at 40 mm. by more than 2-3 mm. Hg; that is to say, the use of the oxygen dissociation curve prepared at a CO_2 tension of 40 ± 2 mm. for the estimation of the oxygen pressure in the maternal venous blood from the placenta appears to introduce a source of error that does not exceed 2-3 mm., an error that would appear to be no greater than those associated with the sampling of the blood in the placental veins. Similar observations on fetal blood indicate that the use of the oxygen dissociation curve prepared at 40 ± 2 mm. Hg of CO_2 pressure for the estimation of the oxygen pressure in the umbilical artery does not introduce any error if the saturations are below about 60, and an error of not more than 2-3 mm. if the

saturations are between 60 and 80 per cent. Hence, I have assumed the pressures so estimated to be representative of the pressures in the umbilical artery.

As an index of the effect of the tissue barrier of the five layered (syndesmochorial) placenta on the transfer of oxygen from the maternal to the fetal blood, I have estimated the coefficient of oxygen utilization of the cotyledons, i.e., that fraction of the total oxygen in the blood of the uterine artery which leaves it through the capillaries of the cotyledons (I have multiplied the fraction by 100 to obtain a whole number):

$$\frac{O_2 \text{ content of uterine artery} - O_2 \text{ content of cotyledonary vessels}}{O_2 \text{ content of artery}} \times 100 = \frac{\text{Coefficient}}{\text{of utilization}}$$

The oxygen that leaves the maternal arterial blood during its passage through the cotyledonary capillaries I have assumed to be taken up by the fetal blood and removed from the cotyledon, and it should be clear that conclusions drawn from these data rest upon the validity of the assumption. But, on the basis of present evidence, it would appear to be justified by the facts for by the fiftieth day of gestation and after, the amount of active maternal tissue in the cotyledon is very small indeed; the prominent structures are the maternal vessels and the fetal villi bearing rich capillary nets on strong cords of Wharton's jelly (Barcroft and Barron[4]).

Results

Oxygen dissociation curves of maternal and fetal blood

The oxygen dissociation curves prepared from the bloods of the six ewes that were not pregnant and had not recently been so, when plotted fell within the area limited by the dotted lines in Figure 1. Of the twenty-four curves prepared with blood from pregnant ewes, twenty lay when plotted within an area on the right margin of the limits for the barren ewes; the other four, Nos. 54, 58, 55, and 60—60, 117, 139, and 140 days' post-insemination respectively—were when plotted still farther to the right and quite outside of the area that included the curves for the barren sheep. The limits of the area that includes all the points on the curves of the bloods of the pregnant ewes are indicated by the solid lines in Figure 1.

All the points on the dissociation curves prepared with bloods from fetuses 111 days' postinsemination and younger when plotted fell within the narrow limits described in Figure 1. The fetal curve characteristic of this period rises rapidly, with but a slight slope to the right from the perpendicular, to a saturation of about 80 per cent; from that level it deviates rather sharply to the right to flatten out and approach the horizontal at a saturation of about 90 per cent. The curves of blood from fetuses in this age range, 50-111 days, are quite to the left of the area characteristic of the barren as well as of that of the pregnant ewes. To produce a saturation of 50 per cent in the blood of the pregnant ewe required an oxygen pressure between 42 and 49 mm. Hg, whereas the blood from fetuses in this age range was half saturated with oxygen at pressures between 17 and 19 mm.

The points on the curves prepared with blood from fetuses 122 days or older all fell to the right of the area characteristic of bloods from fetuses 50-116 days, but some distance to the left of the area enclosing points on the

curves of the bloods of barren ewes (see Fig. 2). The degree to which a particular curve is shifted to the right toward the adult range tends to increase with age of the fetus from which the blood is drawn, but there are exceptions so that the position of the dissociation curve cannot be predicted if the fetus providing the blood is more than 110 days old. This tendency of the curve to shift from the early fetal toward the adult type appears in this series of fetuses to manifest itself first between 116 and 121 days, for the curves prepared from the bloods of fetuses Nos. 52 and 19 (116 and 121

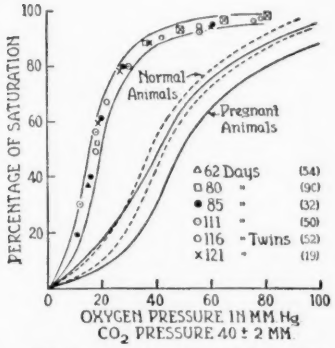

FIG. 1. The fields of distribution of the oxygen dissociation curves of normal barren and of pregnant ewes are limited by the dotted and the solid lines respectively. The individual points represented in the fetal field were determined with blood samples from fetuses whose gestation ages are indicated opposite the appropriate symbols in the key.

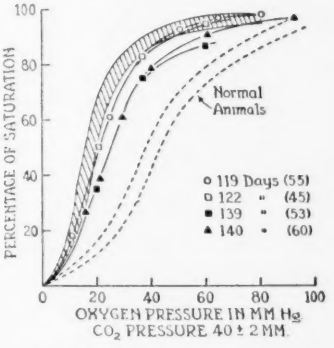

FIG. 2. The oxygen dissociation curves of four fetuses, whose gestation ages are indicated in the key, are plotted together with the field for the curves of some fetuses 120 days and younger to illustrate the shift toward the normal field as gestation advances from 120 days to the end of term—140-145 days. The cross-hatched area represents the area of distribution of the fetal points illustrated in Figure 1.

days respectively) fall within the limits of the early fetal group, whereas the points on the curve of the bloods of No. 55 and No. 45 (119 and 122 days respectively) lie to the right of those for the early fetal group.

At the end of the gestation period the dissociation curve of the fetal blood is still a considerable distance to the left of the limits formed by the curves of blood from adults. The bloods from which the most "advanced" fetal curves were prepared, Nos. 53 and 60, 139 and 140 days' postinsemination respectively—term is usually about 145 days—were half saturated at oxygen pressures of 25-26 mm. Hg, whereas pressures of 38-53 mm. were required to saturate the bloods of barren ewes to the same degree. (See Fig. 2 and Table 2.) The postnatal changes in the character of the oxygen dissociation curves have been followed and are presented in the following paper.

The oxygen pressure gradient between the maternal and fetal bloods

As pointed out above, the oxygen pressure in the blood entering and leaving the placenta was estimated by determining the percentage saturation of a small sample of blood, $\frac{O_2 \text{ content}}{O_2 \text{ capacity}} = \%$ saturation, then locating the point on the dissociation curve prepared with the larger sample of blood, and reading off the oxygen pressure corresponding to that saturation. The

TABLE 2

THE APPROXIMATE OXYGEN PRESSURE IN MM. Hg REQUIRED TO SATURATE THE BLOODS OF BARREN, PREGNANT, AND FETAL SHEEP TO THE DEGREE INDICATED AT CO_2 PRESSURES OF 40 ± 2 MM. Hg

Per cent of saturation	*Barren sheep*	*Pregnant sheep*	*Fetuses 60-120 days*	*Fetuses 120-150 days*
10	14-20	13-24		
20	22-28	22-34	9-13	9-13
40	34-39	36-45	14-18	15-22
60	43-49	46-56	18-20	22-30
80	62-70	66-80	26-28	37-44

data obtained with maternal and fetal bloods from sheep No. 9C sacrificed eighty days after insemination may be used for illustration. The fetal and maternal dissociation curves are illustrated in Figure 3. The oxygen capacity of the maternal blood was 17.3 volumes per cent; the oxygen content of the blood in the uterine artery was 15.2 volumes per cent, i.e., 88 per cent saturated; the point so indicated on the curve in Figure 3 as Ut. Art. corresponds to 80 mm. oxygen tension—the pressure assumed to have existed in the blood taken from the uterine artery. The oxygen content of the sample from the uterine vein was 12.5 volumes per cent, i.e., it was 72.5 per cent saturated, a saturation corresponding on the dissociation curve (Ut. Vein, Fig. 3) to an oxygen pressure of 58 mm. The oxygen capacity of the fetal blood was 12.8 volumes per cent and the oxygen content of the sample from the umbilical vein 11.5, i.e., 90 per cent saturated—a saturation corresponding on the fetal dissociation curve to an oxygen tension of 42 mm. (Umb. Vein, Fig. 3).

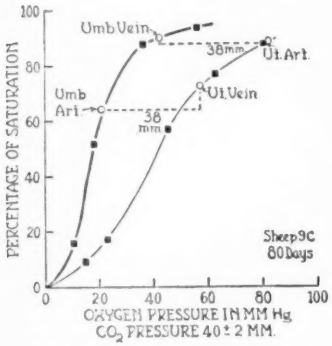

FIG. 3. For explanation see text.

TABLE 3

DATA OBTAINED FROM BLOOD SAMPLES DRAWN FROM THE UTERINE ARTERY (UT.A.), THE UTERINE VEIN (UT.V.), THE UMBILICAL ARTERY (UMB.A.), AND THE UMBILICAL VEIN (UMB. V.).*

Sheep no.	Fetal age Days	Vessel	O_2 cap. Vols. %	O_2 content Vols. %	% Sat.	Est. pO_2	MpO_2—FpO_2
2	56	Ut. A.	13.9	11.22	80.0	75.0	31
		Umb. V.	9.79	9.08	92.7	44.0	
		Ut. V.	13.9	9.93	71.4	62.0	52
		Umb. A.	9.79	2.14	21.9	10.0	
54	62	Ut. A.	13.7	12.8	94.2	90.0	48
		Umb. V.	10.1	4.7	46.5	42.0	
		Ut. V.	13.7	5.3	38.6	39.0	34
		Umb. A.	10.1	00.67	6.6	5.0	
9 gr	65	Umb. A.	12.25	3.56	29.1	14.0	
58–51	76	Umb. A.	10.3	6.84	66.4	22.0	
		Umb. V.		9.86	99.6	70.0	
9C	80	Ut. A.	17.30	15.2	88.0	80.0	38
		Umb. V.	12.76	11.48	90.0	42.0	
		Ut. V.	17.30	12.5	72.5	58.0	38
		Umb. A.	12.76	8.2	64.3	20.0	
23	81	Ut. A.	14.4	13.01	90.0	89.0	59
		Umb. V.	15.6	12.47	80.0	30.0	
		Ut. V.	14.4	11.18	73.3	58.0	39
		Umb. A.	15.6	8.45	54.0	19.0	
8	81	Ut. A.	14.2	12.85	90.8	88.0	50
		Umb. V.	14.1	12.4	87.8	38.0	
		Ut. V.	14.2	9.9	69.8	60.0	40
		Umb. A.	14.1	8.2	58.1	20.0	
32	85	Ut. A.	12.57	11.58	92.1	88.0	50
		Umb. V.	13.31	11.68	88.0	38.0	
		Ut. V.	12.57	8.86	70.4	56.0	38
		Umb. A.	13.31	6.76	52.0	18.0	
5 red	92	Umb. A.	8.9	3.87	43.6	16.0	
		Umb. V.		7.9	89.0	38.0	

* The oxygen capacity (O_2 cap. Vols. %), the oxygen content (O_2 content Vols. %) and the percentage saturation (% Sat.) were used to estimate the oxygen pressure (Est. pO_2) of the sample. The estimated pressure difference at the arterial ends of the parallel placental capillaries is the upper pair of figures in the column MpO_2 — FpO_2; the difference at the venous ends is the lower.

Sheep no.	Fetal age Days	Vessel	O_2 cap. Vols. %	O_2 content Vols. %	% Sat.	Est. pO_2	MpO_2—FpO_2
30	96	Ut. A.	14.9	13.09	87.3	83.0	48
		Umb. V.	15.37	13.2	86.0	35.0	
		Ut. V.	14.9	12.0	80.2	70.0	42
		Umb. A.	15.37	12.25	79.6	28.0	
26	108	Ut. A.	16.8	16.41	97.6	98.0	67
		Umb. V.	12.8	10.73	83.2	31.0	
		Ut. V.	16.8	13.5	80.3	70.0	55
		Umb. A.	12.8	4.48	35.1	15.0	
50	111	Ut. A.	12.2	12.0	98.5	100.0	62
		Umb. V.	12.5	11.0	88.0	38.0	
		Ut. V.	12.2	7.7	63.1	54.0	42
		Umb. A.	12.5	1.4	11.2	8.0	
52	116	Ut. A.	15.5	13.8	89.0	94.0	72
		Umb. V.	15.2	8.8	58.0	22.0	
		Ut. V.	15.5	5.7	36.7	39.0	25
		Umb. A.	15.2	4.6	30.2	14.0	
55	119	Ut. V.	14.3	10.5	73.5	60.0	40
		Umb. A.	11.2	7.0	62.5	20.0	
5	120	Ut. A.	19.2	19.1	99.5	100.0	78
		Umb. V.	16.3	8.45	51.8	22.0	
		Ut. V.	19.2	9.0	46.8	44.0	36
		Umb. A.	16.3	1.84	11.3	8.0	
58	121	Ut. A.	12.8	11.0	86.0	87.0	67
		Umb. V.	15.7	7.3	46.5	20.0	
		Ut. V.	12.8	3.8	29.7	38.0	24
		Umb. A.	15.7	5.0	31.8	14.0	
19C	121	Ut. A.	16.7	14.0	84.0	84.0	64
		Umb. V.	19.0	14.2	46.0	20.0	
		Ut. V.	16.7	9.8	48.0	58.0	48
		Umb. A.	19.0	4.0	21.0	10.0	
24	122	Ut. A.	12.73	10.8	85.0	86.0	65
		Umb. V.	18.2	9.0	49.6	21.0	
		Ut. V.	12.73	8.91	70.0	58.0	42
		Umb. A.	18.2	5.39	29.5	16.0	
68	126	Ut. A.	12.82	10.65	83.4	80.0	62
		Umb. V.	10.70	4.15	38.8	18.0	
		Ut. V.	12.82	7.65	59.6	50.0	36
		Umb. A.	10.70	2.68	25.0	14.0	

Sheep no.	Fetal age Days	Vessel	O_2 cap. Vols. %	O_2 content Vols. %	% Sat.	Est. pO_2	MpO_2—FpO_2
70	126	Ut. A.	14.9	13.0	87.4	86.0	62
		Umb. V.	18.35	10.5	57.3	24.0	
		Ut. V.	14.9	7.49	50.3	46.0	31
		Umb. A.	18.35	6.46	35.2	15.0	
3	136	Ut. A.	13.7	12.4	90.5	92.0	54
		Umb. V.	15.0	11.7	78.0	28.0	
		Ut. V.	13.7	9.25[1]	67.4	58.0	34
		Umb. A.	15.0	7.22	48.1	24.0	
11	136	Ut. A.	15.4	13.5	85.8	82.0	51
		Umb. V.	15.5	10.1	65.21	31.0	
		Ut. V.	15.4	9.30	60.4	52.0	31
		Umb. A.	15.5	6.98	45.0	21.0	
19	137	Ut. A.	13.7	11.9	87.0	84.0	57
		Umb. V.	20.6	11.5	54.1	27.0	
		Ut. V.	13.7	7.8	57.0	50.0	33
		Umb. A.	20.6	6.6	32.0	17.0	
53	139	Ut. A.	18.16	15.6	86.0	82.0	56
		Umb. V.	16.67	8.3	49.6	26.0	
		Ut. V.	18.16	12.9	71.0	58.0	40
		Umb. A.	16.67	4.8	29.0	18.0	
60	140	Ut. A.	12.8	11.3	88.2	86.0	50
		Umb. V.	16.6	12.1	73.0	36.0	
		Ut. V.	12.8	6.6	51.6	46.0	33
		Umb. A.	16.6	3.4	20.4	13.0	

The oxygen content of the sample from the umbilical artery was 8.2 volumes per cent, i.e., it was 64.3 per cent saturated (Umb. Art., Fig. 3). Reference to the fetal dissociation curve prepared with this same blood indicates that at this stage in development an oxygen pressure of 20 mm. is required to saturate the blood to that degree (Umb. Art., Fig. 3). As the blood leaving the placenta via the umbilical vein is in equilibrium with blood entering through the uterine artery, the difference of 38 mm. in the oxygen pressures of the bloods of these two vessels represents the gradient at the arterial ends of the parallel capillary cords in the placenta, while the difference in oxygen pressure in the bloods of the uterine vein and umbilical artery—in this case also 38 mm.—typifies the gradient at the venous ends of the capillary cords.

The results obtained by treating in this manner each one of the blood samples obtained from the twenty-five pregnant sheep are presented in Table 3. The upper figure in the column headed MpO_2 — FpO_2 (the oxy-

gen pressure in the maternal vessel — the oxygen pressure in the opposing fetal one) is the estimated oxygen pressure difference at the arterial ends of the placental vessels; the lower figure is the pressure difference at the venous ends. These differences in pressures as well as the pressures in the two umbilical vessels are represented graphically in Figures 4 and 5 respectively.

TABLE 4

COEFFICIENT OF OXYGEN UTILIZATION BY COTYLEDONS OF THE UTERUS OF THE GRAVID SHEEP

Sheep no.	*Fetal age in days*	*O_2 cap. Vols. %*	*Oxygen content vols. %*		*Difference*	*Coefficient of utilization*
			Ut. Art.	*Ut. Vein*		
			Singlets			
59	50		14.20	11.77	2.43	17.1
2	56	13.9	11.22	9.92	1.29	11.5
54	62		16.15	12.35	3.80	23.5
9C	80	17.3	15.2	12.5	2.7	17.8
23	81	14.4	13.01	11.18	1.82	14.0
32	85	12.57	11.58	8.86	2.72	23.5
30	96	14.9	13.09	12.0	1.09	8.3
26	108	16.8	16.41	13.5	2.91	18.2
50	111	12.2	12.0	7.7	4.3	35.8
19C	121	16.7	14.0	9.8	5.2	37.2
24	122	12.73	10.8	8.9	1.9	17.6
68	126	12.82	10.65	7.65	3.0	28.2
3	136	13.7	12.4	9.25	3.15	25.4
11	136	15.4	13.5	9.30	4.15	30.7
53	139	18.16	15.6	12.9	2.7	17.3
60	140	12.8	11.3	6.6	4.7	41.5
			Twins			
9	50	17.35	14.38	10.25	4.13	28.8
8	81	14.2	12.85	9.9	2.9	22.6
52	116	15.5	13.8	5.7	8.1	58.7
5	120	19.2	19.1	9.0	10.1	52.9
58	121	12.8	11.0	3.8	7.2	65.5
70	126	14.9	13.0	7.49	5.51	42.5
19	137	13.7	11.9	7.8	4.1	34.4

The coefficient of oxygen utilization

The oxygen contents of the bloods of the uterine artery and vein of the ewes in the series used for the estimation of the placental oxygen pressure gradient are repeated in Table 4, which includes data from two other sheep. The difference in the oxygen contents of these two vessels, which represents the fraction of the oxygen lost to the uterine tissues and the fetal blood—a relatively small proportion appears to be utilized by the former—is used

together with the oxygen content of the uterine artery in the calculation of the coefficient of utilization. The data from the individual ewes are presented in two subdivisions of the table; the upper includes those based on the results of the ewes that had singlets, the lower the data derived from the blood of ewes with twins. The average figures given for the oxygen content of the uterine artery, the difference in the oxygen contents of the artery and vein, as well as the coefficient of utilization are included to be used later for purposes of discussion simply as representative or characteristic values.

Discussion

In addition to the purpose they serve in the estimation of the oxygen pressure across the placenta, the observations presented here on the characteristics of the dissociation curves of fetal and maternal sheep blood provide a basis for some inferences with regard to the changes that may occur in the blood of an individual fetus with advancing development, as well as those changes occurring in the blood of the pregnant ewe during the course of gestation; moreover, they permit a comparison of the dissociation curves with those of other species having syndesmochorial placentae, i.e., the goat (Barcroft *et al.*[5]) and the cow (Roos and Romjin[22, 23]).

As the curves prepared with bloods of fetuses of 116 days and younger all fall within the same narrow limits, the data suggest that if curves were prepared with the blood of a single fetus at successive stages in development, they would be similarly uniform in their contour and position until about 120 days; after that date—allowing a few days either way for individual variation—the data indicate that curves prepared with blood from this same fetus, as gestation advances, would lie progressively farther to the right and become more sigmoidal in character to approach the position and contour of curves of blood from normal mature sheep. This suggestion is supported by observations at regular intervals on the dissociation curves of bloods of individual lambs delivered by caesarian section (Barron[2]).

The shape and the position of the dissociation curve of the fetal blood prior to about 120 days appear to be due to the presence in the corpuscles of a haemoglobin that differs from that found in adult corpuscles in at least two features. These features are: (i) its solubility (Karnoven[18]) and (ii) the form of its oxygen dissociation curve when prepared with dilute solutions (Hill[16]). The solubility of the haemoglobin of fetuses older than 115-120 days Karnoven found could be duplicated by mixing appropriate amounts of haemoglobin of adults with that of fetuses younger than 115 days. The shift of the dissociation curve to the right in the final month of gestation appears to be due to the appearance of the adult type of haemoglobin in increasing amounts and a concomitant decline in the proportion of the fetal type. Whether this adult haemoglobin is confined to specific red cells and the fetal type to others, or whether a mixture of both is to be

found in any single cell, the data at hand do not suggest; but clearly the adult type of haemoglobin makes its first appearance at a fairly specific stage in the development of the sheep fetus.

The stable character of the dissociation curve of the fetal sheep's blood during the greater part of the gestation period, as well as the position relative to the maternal, contrast rather sharply with the corresponding aspects of the curves of the fetal blood of the kid—a closely allied species. Barcroft and his collaborators[5] found that the points determined with bloods of the youngest fetal kids, aged 10 weeks and 10 weeks and one day respectively, fell in the area including curves of normal animals; with bloods from individuals at more advanced stages in gestation—13 weeks 3 days, and 14 weeks one day—the points at the higher saturations, *circa* 70 per cent, remained within the adult field or close to it, whilst the points at lower saturations—50 per cent and less—were to the left of the adult field, i.e., there was a tendency for the fetal and adult curves to cross at higher saturations. With bloods of individuals still later in gestation these points at higher saturations moved to the left so that the fetal curve lay just to the left of the adult; earlier the curve tended to be hyperbolic but as the term was approached it was definitely sigmoidal and might lie within the field of the adults.

These differences between the curves of the fetal sheep and the goat are the more striking when they are considered together with the observations of Roos and Romjin[22, 23] on the blood of the fetal cow. Their series is not large but their curves for the blood of the youngest fetal calves—3 and 3½ months respectively—are very similar in position and contour to those described here for the fetal sheep up to *circa* 115-120 days; the transition, in position and contour, toward the adult type of curve appears to be underway in the fetal calf at 5½ months of gestation age and to progress—with due allowance for individual variations—until birth, though it is not complete at that time. At the moment no explanation is at hand which will account for the apparent regularity with which the dissociation curves of the fetal lamb and calf change during gestation and the apparent absence of such regularity in the blood of the fetal goat, but it may be that these differences will be found associated with differences in the period in development at which the adult type of haemoglobin appears in fetal blood and the circumstances that govern its production. In contrast to that of the fetal, the position as well as shape of the maternal oxygen dissociation curve appear to remain relatively unchanged throughout the entire gestation period; the data indicate that there may be in some individuals a tendency for the dissociation curve to be shifted to the right of the area including curves for barren sheep but it is clear that the shift does not occur *pari pasu* as gestation advances nor is it characteristic of any interval in the gestation period.

As the type of haemoglobin in the maternal corpuscles is unaltered in pregnancy (Barcroft[3]) the data permit the further inference that there is no alteration in the hydrogen ion concentration of the maternal blood in pregnancy—no pregnancy acidosis—such as Barcroft *et al.*[5] described in pregnant goats. This inference is supported by a large series of observations on the pH of the blood of pregnant sheep made at regular fortnightly intervals before, during, and after their pregnancy (Barron, unpublished). In this respect, too, the circumstances in pregnant sheep resemble those in the pregnant cow; the dissociation curve of the blood of the pregnant cow falls within the range for normal barren animals. Though it may be no more than coincidence, it is of interest that in these two species in which the maternal dissociation curve appears to be unaltered by pregnancy, the fetal curve is placed far to the left of that of the normal adult; in the goat, the fetal curve lies relatively near the region of the adult, but because of the shift in the position of the maternal curve the fetal and maternal are widely separated; the separation of the two curves, fetal and maternal, whatever the mechanism by which it is wrought, serves to promote the transfer of oxygen from the maternal blood to the fetal.

The comparative studies of Hall and his collaborators[15] on the oxygen dissociation curves of the bloods and haemoglobins of birds and mammals indigenous to terrain at high altitudes where the oxygen pressure in the atmosphere is reduced have shown that the curves of these bloods tend to be placed to the left of those for bloods of closely allied species with a native habitat at or near sea level—an obvious adaptation to the environment. The position of the fetal curve suggests then, in accordance with Hall's observations, that the oxygen pressure to which the fetal blood is exposed is likewise relatively low—a suggestion that is born out by the data at hand, for oxygen pressures of 55 and 56 mm. were the highest that were observed in the arterialized blood of the umbilical vein draining the cotyledons of the placenta. In fact, oxygen pressures of more than 40 mm. were only found in the umbilical vein bloods of the fetuses of 80 days' gestation age or younger; the pressures in the arterialized blood of fetuses 81 to 111 days ranged between 30 and 38 mm. In older fetuses up to 126 days' gestation age the pressures were still lower, ranging between 32 and 34 mm. Hg; the oxygen pressures in the arterialized blood of the fetuses in the final week of the gestation period, 136-140 days, were slightly higher than those in younger fetuses. The range was between 36 and 46 mm. Hg.

Further, these data indicate quite clearly that the oxygen pressure in the blood of the fetal umbilical vein tends to be within particular ranges at different stages of gestation; and it follows that if the oxygen pressure in the blood of the umbilical vein of an individual fetus could be followed through the portion of the gestation period under consideration here, it would vary in a similar manner as gestation advances. These variations in oxygen pressure as they occur in a single fetus might be represented by

the smoothed curve in Figure 4; and if this curve be accepted as a first approximation of the pressure variations with time, some inferences may be drawn with regard to the circumstances responsible for these variations.

The data at hand provide no evidence that the variations in oxygen pressure of the blood leaving the cotyledons are due to or associated with variations in the oxygen pressure in the maternal arterial blood; the sheep appears to ventilate poorly when placed on its back, and there are wide variations from individual to individual in the oxygen level of the arterial blood on the maternal side but they do not appear to be correlated with the changes on the fetal side. Nor do these changes in the oxygen pressure in the fetal blood appear to be correlated with the changes in the organization of the vascular beds of the placenta; the period up to 80-90 days' gestation age during which the oxygen pressure is highest corresponds to the period during which the maternal tissues are erroded and the final structure of the placenta, i.e., its syndesmochorial character, is established and in which the fetal vascular bed in the placenta approaches its maximum development (Barcroft and Barron[4]; see also Barcroft,[3] p. 74). Thereafter, the proximity of the fetal and maternal capillaries and the surface area of the fetal capillary bed appear to remain substantially unchanged until the end of term. Hence, the fall in oxygen pressure in the umbilical vein blood from the 80th day of gestation onward does not appear to be referable to changes in the physical features of the placenta, and there remains to be explored the possibility that the fall in pressure is due to circumstances that result from the development of the fetus itself.

During the first 80 days of the gestation period, the fetus increases in weight to about 200-250 grams; in the next forty days the weight increases sevenfold (Barcroft,[3] p. 33). If the oxygen requirements of the fetus increase in approximately the same proportion as the weight, then they too will have risen sevenfold despite the fact that the fetal capillary surface through which oxygen can enter the fetal blood will remain virtually unchanged. Now the forces that determine the quantity of oxygen (cc.'s per minute) that will diffuse from the maternal to the fetal blood across the placenta of an 80-day fetus are: (i) the difference in oxygen pressure in the bloods in the two sets of capillaries; (ii) the size of the capillary bed with the smaller surface area; and (iii) the resistance offered to the movement of the gas by the maternal and fetal capillary walls together with the tissues that separate them.

These forces can be related and expressed as follows:

$$Q = \frac{(P_m - P_f) \times C_a}{R}$$

where Q = cc.'s of oxygen transferred per minute from the maternal to the fetal blood; P_m and P_f represent the average oxygen pressures in mm. Hg in the maternal and fetal capillaries respectively; C_a the surface

area of the smaller of the two capillary beds in square mm.—in this case apparently the fetal; and R the resistance offered by the tissues separating the two bloods.

As the fetus grows in the period after 80 days its oxygen requirements increase, that is to say, Q must increase apace, but P_m appears to be fixed; so too C_a, the surface area for diffusion, and R the resistance offered by the tissues. P_f appears to be the only factor on the right side of the equation subject to any appreciable change as gestation advances; a reduction in P_f would, other things being equal, increase the quantity of oxygen transferred per unit time and serve to meet the demands of the fetus.

Thus far in the discussion of this point, I have presented evidence that the oxygen pressure does drop in the umbilical vein after about 80 days and that such a drop would serve to increase the fetal oxygen supply; indeed, it appears to be the only device by which the oxygen demands of the growing fetus could be met. If now one could show that during this period the fetal circulation is adjusted to produce such a drop in the oxygen pressure of the fetal blood as it leaves the placental capillaries, the thesis would be strengthened. The evidence on this point is as follows.

The oxygen pressure in the fetal capillaries of the placenta will depend, other circumstances remaining the same, on the rate at which the oxygen arriving there is removed by the fetal blood, and that rate will depend in turn on the rate of blood flow through the fetal capillaries and the amount of oxygen removed per unit volume. The beautiful studies of Cooper, Greenfield, and Huggett[7] (see their Fig. 1, p. 162) demonstrate the tremendous increase in umbilical-placental blood flow that occurs as the age of the fetus studied moves past the 80th day toward term—from around 50 cc.'s per minute at 80 days to something in the neighborhood of 300-350 cc.'s near full term. This increase in blood flow through what appears to be a vascular bed with its surface area fixed at about 80 days, together with the steady rise that occurs in the oxygen capacity of the fetal blood, would appear to provide the mechanisms for the reduction of the oxygen pressure observed in the fetal capillaries and so to promote the transfer of oxygen from the maternal to the fetal blood.

In passing it may be worthwhile to point out that the sequelae of the procedures essential to the operative delivery of the umbilical vessels and for withdrawing the blood samples are those that would tend to lead to uterine contraction and so to a reduction in the rate of flow of blood through the fetal and maternal capillaries; such a reduction in flow should serve to increase the oxygen pressure in the fetal blood leaving cotyledons and so to reduce the gradient in pressure between the oxygen in the maternal arterial and fetal arterialized blood. If this reasoning is sound, it would appear that the lower pressures in the umbilical vein and the higher pressure differences between maternal and fetal bloods may be more representative of actual conditions in the intact uterus than are the lower.

The oxygen pressure of the blood in the umbilical artery is, of course, determined by circumstances in the fetus. In accordance with expectation, the data at hand indicate they are lower than those in the corresponding samples from the umbilical vein. Moreover, in these pressures there appears to be a variation that is associated with the ages of the fetuses studied; the pressures are low in the youngest fetuses, ranging between 5 and 13 mm. Hg for those between 56 and 65 days' gestation age. Between 74 and 92 days the pressures are higher, 18 to 22 mm.; between 108 and 126 days they vary between 8 and 16 mm. At the end of gestation, 136 to 140 days, the pressures tend to be higher, 17-24 mm. These variations with age are

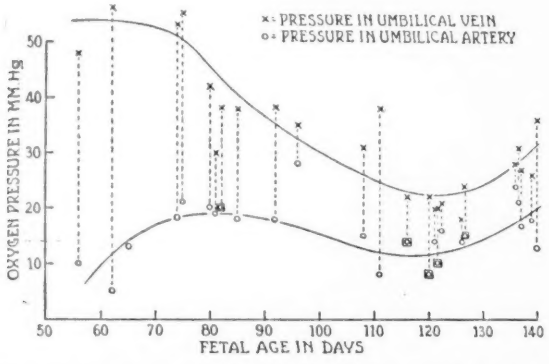

FIG. 4. The oxygen pressures in the blood of the umbilical vessels in fetuses at several stages in gestation as determined by means of the oxygen dissociation curve and the percentage saturation. The smoothed curves illustrate the probable changes in oxygen pressure in the blood entering and leaving the placenta of an individual fetus as gestation advances. The square about the circle marking the pressure in the umbilical vein indicates the individual was a twin.

so small relative to the pressure changes in the blood of the umbilical vein that they might at first glance appear to represent the variations associated with the technique of sampling, but it must be regarded as more than coincidence that similar fluctuations associated with the corresponding fetal ages are to be found in Barcroft's data[3] (p. 146) on the percentage saturation of the blood in the umbilical artery in his series of fetuses. If these saturations are used to estimate oxygen pressures, employing dissociation curves illustrated here for the appropriate fetal ages, the pressure variations with advancing age show the same trends as those illustrated in Figure 4. Accordingly, as a first approximation one may not be too far from the mark if he assumes that the variations in pressure with advancing age in an individual fetus could be represented by the smoothed curve in Figure 4.

If the variations in the oxygen pressure in the umbilical artery do correspond in any one fetus to the smoothed curve, the question follows:

"What are the circumstances in the fetus that are responsible for them?" At the moment no final answer can be given; they might be due to variations in the oxygen utilization of the fetal tissues, but there is a greater likelihood that they are associated with fluctuations in the fraction of the arterialized blood, returning from the placenta via the inferior vena cava to the heart, that is diverted into the right ventricle to find its way back to the aorta through the ductus arteriosus. The greater that fraction is relative to the superior caval return, the higher the oxygen pressure in the umbilical artery will tend to be. When the fraction is a constant one and the oxygen utilization by the upper circulation represented by the superior caval return is constant, then the oxygen pressure in the umbilical artery will tend to follow but be below that in the umbilical vein.

The data presented here further indicate that the difference in pressure ($P_m - P_f$) required to provide for the transfer of oxygen across the tissue barrier of the placenta at the rate it is utilized by the developing fetus is of the order of 40 to 50 mm. Hg from about 80 days' gestation age onward until the end of term—a pressure difference similar to that found by Huggett[17] (the first to investigate the subject) to exist across the capillaries of the syndesmochorial placenta of the near term goat; by Barcroft,[3] and Roos and Romjin,[24] in their studies in the sheep and the cow respectively; and in the older members of a small series of nine cases I reported earlier (Barron[1]) at 97-147 days. In the younger fetuses in that series the differences I found to be of the order of 11 to 23 mm., but these values appear to be too low due to the fact that the dissociation curve used for the estimations of the pressures in the fetal blood was not characteristic of the stage represented—a fact that became evident only after I had prepared other curves from the bloods of fetuses in this age range. Barcroft estimated the gradient in two sheep, one 111 days, the other 152 days; Roos and Romjin's five cows were in the last two months of the gestation period. The results of Barcroft and of Roos and Romjin are of further interest for they did not attempt to estimate the average pressure gradient but instead the pressure gradients at each end of the parallel cords of the maternal and fetal capillaries. Here again there is agreement between their observations and my own, for they found the pressure difference to be greater at the arterial ends of the parallel cords of capillaries than at the venous.

More specifically, the data presented here indicate that the pressure gradient is steeper at the arterial ends of these capillary cords than at the venous by an amount that varies between 0 and 47 mm. Hg in the fetuses 80 days' gestation age or older. The greatest differences in the gradient pressures at the two ends were found in sheep Nos. 52, 5, and 58 (116, 120, and 121 days' gestation age respectively). Each of these ewes bore twins. The data are not sufficient to establish the point but there is the suggestion in them (see Fig. 5) that in those twins sacrificed in the last third of gestation, the pressure difference at the venous ends of the capillaries is

less than that characteristic of ewes bearing singlets, whereas it is greater at the arterial ends in the same circumstances. The factors that result in the difference in the slope at the two ends of the capillary cords have yet to be discovered but they will be found to be associated, in all probability, with the rate at which the fetal and maternal bloods move past each other through the placental capillaries.

In any case the slope of the gradient at the venous ends of the capillaries is of prime importance to the mother, for it serves to limit the fraction of the oxygen content of each cc. of arterial blood arriving via the uterine

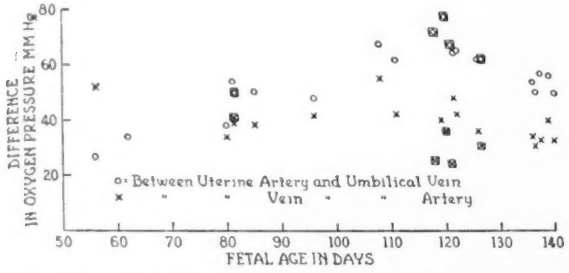

FIG. 5. The differences between the oxygen pressures in the blood of the uterine vein and the umbilical artery, and between those in the blood of the uterine artery and the umbilical vein, as observed in fetuses at several stages in gestation, are arranged to illustrate the trends in these differences as development advances and those that might be expected to occur during the development of an individual fetus. The square around the circle indicates the individual was one of twins.

artery that can be transferred to the fetus; the other factor is of course the oxygen pressure in the umbilical artery. Their importance would appear to be reflected in coefficients of utilization of the placenta as presented in Table 4. Despite a certain ruggedness, the data in that table do indicate that the ewe with a singlet is able to transfer to the fetus only a small fraction of the total oxygen brought to the placenta by each cc. of her blood and that the ewes with twins are more efficient in respect to this transfer; in other words, they transfer a larger fraction of the oxygen brought to the placenta. For what it is worth the average of the utilization coefficients for ewes with singlets is 23.2 per cent and for the ewes with twins 44.0 per cent. These figures are fairly representative at least; that is to say, they illustrate the order of magnitude of the coefficient one might expect to obtain if he were to do another experiment, and they serve as useful figures for the extension of one's thinking about the oxygen transfer in the placenta of the sheep along the following lines.

The oxygen capacity of a pregnant ewe may be fairly represented as 15.0 volumes per cent, the oxygen pressure in the fetal blood returning to the placenta—umbilical artery—as 16 mm. Hg, and the oxygen pressure gradi-

ent between the blood in the uterine vein and that of the umbilical artery as 45 mm. On this basis the oxygen pressure in the uterine vein could not be less than 61 mm. Hg and at that pressure the ewe's blood is about 70 per cent saturated. The arterial blood of the ewe in the circumstances of my experiments is of the order of 90 per cent saturated. Hence, the maternal blood could give up to the fetus 20 per cent of its oxygen load—a figure not too far removed from the average 23.2 per cent given above—or 2.7 cc.'s of oxygen for each 100 cc.'s of maternal blood flowing through the placenta. Further, Barcroft's[3] figures indicate that the fetal oxygen consumption is of the order of 7.6 cc.'s per minute per kilo near the end of term. If the fetus weighed 2.5 kilos with an O_2 consumption of, say, 19.5 cc.'s per minute, the ewe would, on the basis of these figures used above, be obliged to put some 720 cc.'s of blood per minute through the placenta to meet the fetal demands.

The results of such calculations, though they may be wide of the mark in any individual case, probably represent a first approximation to the circumstances associated with the oxygen transfer from mother to fetus in the ewe and they do indicate what might be done on a comparative basis when satisfactory data are available; the research described here is being extended in a search for them.

Summary

To evaluate the rôle of the tissue layers of the syndesmochorial placenta of the sheep in the movement of oxygen from the maternal to the fetal blood, the oxygen pressure gradient between the two bloods has been estimated at both ends of the parallel cords of placental capillaries by relating the percentage saturation of the samples drawn from the appropriate vessels —uterine artery, uterine vein, umbilical artery, umbilical vein—to the oxygen dissociation curve prepared from the same blood or one of similar characteristics. The range of variation in oxygen dissociation curves of normal ewes and of pregnant ewes has been determined—the latter range overlaps the normal on the right margin to extend beyond it; the curves of fetal blood from fetuses younger than 116 days are to the left of the normal field. Curves of older fetuses tend to lie progressively farther to the right as gestation advances but are still short of the normal field at full term. The oxygen pressure gradient between the fetal and maternal blood tends to be greater at the arterial than at the venous ends of the parallel cords of placental capillaries over the last 60 days of gestation; and this difference tends to be greater in ewes with twins than in those with singlets. The pressure difference at the venous ends is of the order of 38-45 mm. Hg. As the oxygen pressure in the umbilical artery is of the order of 16 to 20 mm., the unloading pressure of the uterine vein is the order 54 to 65 mm. Hg, a tension at which the maternal blood is still about 70 per cent saturated. Estimations of the coefficient of oxygen utilization by the uterus are com-

patible with these findings and range from 8.3-41.5 in ewes with singlets to 22.6-65.5 in ewes with twins. The data presented are used to estimate the uterine flow necessary to provide for the needs of the fetus.

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POSTNATAL CHANGES IN THE OXYGEN CAPACITY AND DISSOCIATION CURVE OF THE BLOOD OF LAMBS*

DONALD H. BARRON

Observations on the form and position of the oxygen dissociation curve of the bloods of fetal sheep indicate that they are uniform in character and position from the 60th through the 116th day postinsemination, though placed far to the left of similar curves prepared with bloods from normal adults.[1] Curves prepared with bloods from fetuses 117-121 days postinsemination may be of the earlier form and position or be placed slightly to the right, i.e., the period marks the beginning of the transition from the earlier fetal toward the adult type of dissociation curve. The curves of bloods of older fetuses, 122nd day postinsemination to the end of gestation at about 145-150 days, appear always to be situated to the right of the earlier curves characteristic of the first four months of the gestation period, but the most advanced curve obtained from the blood of a full-term fetus delivered by caesarian section was still far to the left of the limits of the area in which the curves of normal adults fall. Clearly the transition from the fetal type of oxygen dissociation curve to that of the adult sheep must be completed in the postnatal period. The object of the experiments to be reported here was to determine: (i) the rate at which this transition takes place postnatally; (ii) when it is completed; and (iii) to discover circumstances that might be associated with its genesis and rate.

Materials and Methods

For these experiments eight lambs were available; two, Nos. 19c and 55, were delivered by caesarian section at 121 and 139 days postinsemination, respectively. The other six lambs, Nos. 56, Betsy, Patrick, Bagdad, Finise, and Mary, were delivered naturally in the laboratory at full term. Nos. 19c and 55 were bottle fed on cows' milk; Bagdad was similarly fed after the mother died six days postpartum. The other lambs were nursed by their mothers. All of the lambs, except Bagdad, appeared to gain in weight and to develop normally. Bagdad was always smaller and less strong than the others. Except for the bloods that were taken from the umbilical vessels for the initial curves of the two lambs which were delivered by caesarian section, the samples were drawn from the external jugular vein, without anesthetic. They were drawn into a well-oiled syringe in which the dead space was filled with a solution of oxalate and fluoride (3 grams potassium acid oxalate and 1 gram sodium fluoride in 100 ml. distilled water) and contained sufficient in excess so that the final sample was diluted one part of solution to nine of blood.

Five cc. samples were equilibrated at 39° C. in 250-300 cc. tonometers containing gas mixtures in which the oxygen tensions had been adjusted to appropriate levels by re-

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placing the air in them with nitrogen; CO_2 was added so that the pressure at equilibrium was 40 ± 2 mm. Hg. The pressure in the tonometers was brought to atmospheric by opening the stopcocks two or three millimeters below the surface of the water and allowing the excess gas to escape (about 5-8 minutes after their introduction into the bath). The blood was withdrawn from the tonometer, by a needle thrust through its rubber stopper, into a well-oiled syringe which served to store the sample before it was transferred to the Van Slyke pipette. The gas content of the bloods was determined in duplicate on 0.5 cc. samples by the manometric method of Van Slyke. The gas analyses were done in duplicate by means of the Henderson-Haldane apparatus.

Results

Within this relatively small series of six lambs born naturally, at "full term," there is a wide variation in the position of the oxygen dissociation

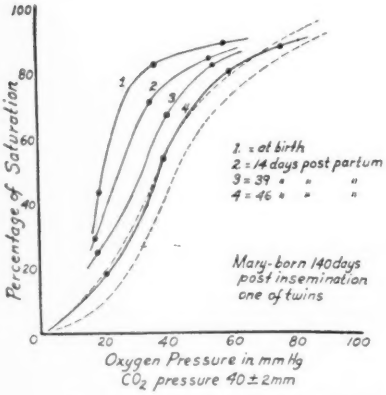

Fig. 1. Oxygen dissociation curves prepared with blood drawn from the lamb Mary at intervals as indicated. The field occupied by curves prepared with bloods of normal adults is limited by the dashed lines.

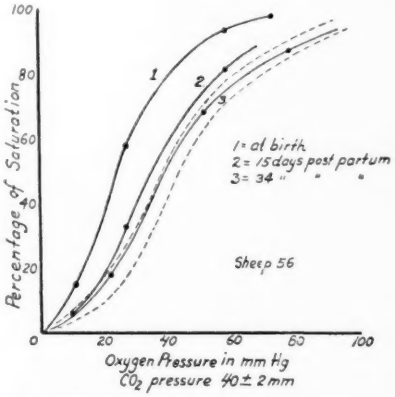

Fig. 2. Oxygen dissociation curves prepared with blood from lamb of sheep 56. Bloods for curves were drawn at the intervals indicated; the dotted lines enclose the field occupied by curves prepared with blood of normal adults.

curves of individual bloods taken at birth. The two extremes in this range of variation are represented by the positions of the curves obtained with the bloods of the lambs Mary (Fig. 1) and sheep 56 (Fig. 2). The position of the curve of the blood of Mary is just slightly to the right of the field which includes the curves of bloods of fetuses 60 to 120 days postinsemination,[1] whereas the position of the curve of the blood from the lamb of sheep 56 is similar in shape and position to curves of fetuses of 140-150 days delivered by caesarian section. The curves of the bloods of the other four lambs are intermediate in position; that of the blood of Betsy is just to the right of the curve of Mary's blood and it is of the same general form. The curves of the other three lambs were similar in form and grouped close to the curve of the lamb of sheep 56. Though the factors which determine the

position of the dissociation curve at birth are unknown, it may be significant that Mary and Betsy both had litter mates, i.e., they were the individuals selected for study from two sets of twins. The other four individuals whose bloods were studied were singlets. All of the lambs ranged between 3.8-4.2 kilos at birth and made satisfactory weight gains in the neonatal period—Bagdad excepted.

A comparison of the dissociation curves of bloods taken from an individual lamb at approximately weekly intervals after its delivery—either naturally or by caesarian section—indicates that (Figs. 1, 2, 3, and 4) the

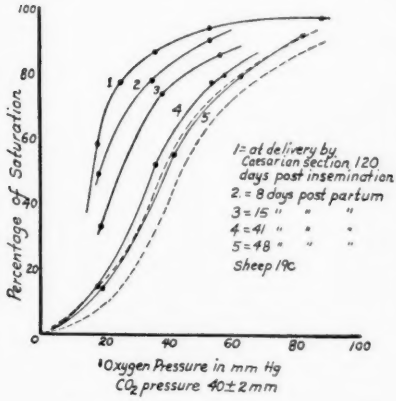

FIG. 3. Oxygen dissociation curves prepared with blood from lamb of sheep 19c; the bloods were drawn at the intervals indicated. Again the normal adult field is represented by the dotted lines.

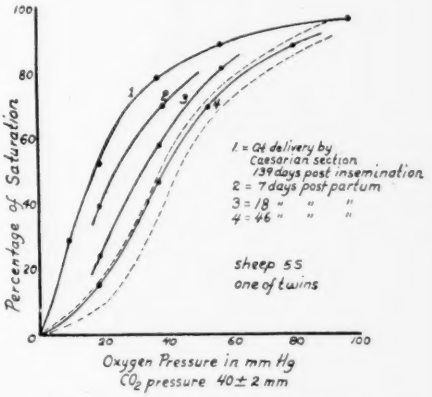

FIG. 4. Oxygen dissociation curves prepared with blood from the lamb of sheep 55; the bloods were drawn at the intervals indicated. The normal adult field is represented by the dotted lines.

rate of change in the blood from the fetal or natal toward the adult character, as indicated by the form and position of the curves, is most rapid in the fortnight immediately following delivery. Thereafter the dissociation curve shifts more slowly toward the right to reach the field for adult sheep between 34 (sheep 56) and 48 days (sheep 19c) after delivery. The interval required for the completion of the transition appears to be determined by the position of the dissociation curve at birth; the farther the curve is toward the right or adult field at birth, the shorter the postnatal period of transition.

In the fortnight immediately after birth, when the dissociation curve is shifting most rapidly toward the right, there is usually a marked reduction in the oxygen capacity of the blood. All of the six lambs born naturally had oxygen capacities that were higher than that of their mothers on the day of delivery by amounts ranging from 0.4 to 3.1 volumes per cent. In three of the four lambs in which the oxygen capacities were examined during the

three or four days immediately after birth, the oxygen capacity of the blood rose 0.4 to 1.5 volumes per cent above the value at birth before the decline. A decrease in oxygen capacity which occurs in all the individuals of the series ceases between eleven and twenty-one days after birth (the lamb Bagdad may be an exception); this minimal value reached ranges between 86.2 and 45.8 per cent of the original capacity in this group of lambs (see Fig. 5).

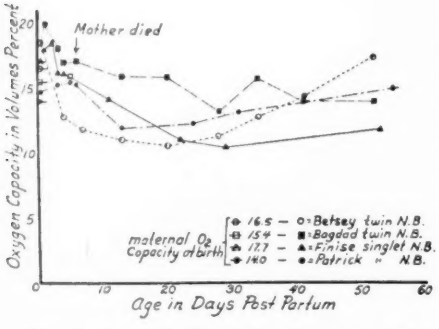
Oxygen Capacity in Volumes Percent
Mother died
maternal O2 Capacity at birth
16.5 — o = Betsey twin N.B.
15.4 — ■ = Bagdad twin N.B.
17.7 — ▲ = Finise singlet N.B.
14.0 — ● = Patrick " N.B.
Age in Days Post Partum

FIG. 5. A graph illustrating the postoperative changes in oxygen capacity of the blood of lambs born naturally (N.B.).

This decrease in the oxygen capacity of the blood in the fortnight or three weeks after delivery appears to be due to a decrease in volume of circulating red cells, for observations on the concentration of the plasma proteins by the falling drop method of Barbour and Hamilton[2] (see Fig. 6) provide no evidence that the decrease is due to hemal dilution. At birth the concentration of the plasma proteins ranged between 3.4 and 6.8 grams per 100 cc. In some individuals the concentration remained unchanged during the first twenty-five days after birth; in others it fell, e.g., from 6.8 to 6.12 grams per 100 cc. in the lamb Mary. The concentration rose in some individuals as it did in the plasma of lamb 19c from 4.1 to 5.4 grams per 100 cc.

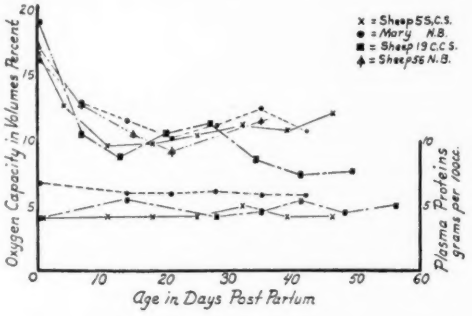
x = Sheep 55, C.S.
● = Mary N.B.
■ = Sheep 19 C.C.S.
▲ = Sheep 56 N.B.
Oxygen Capacity in Volumes Percent
Plasma Proteins grams per 100cc.
Age in Days Post Partum

FIG. 6. A graph illustrating the postoperative changes in the oxygen capacity of the blood of lambs born by caesarian section (C.S.) and naturally (N.B.) together with the changes in the protein concentration in the plasma.

DISCUSSION

These observations on the rate of change in the position of the oxygen dissociation of lambs after birth are in agreement with the solubility studies of Karvonen[3] on the changes in the character of the haemoglobin. According to these studies the transition which begins from about 120 days postinsemi-

nation—at that time the haemoglobin is 100 per cent of the fetal type—occupies two to three months before the haemoglobin of the blood is all of the adult type, i.e., the transition is already in progress at full term, 145-150 days postinsemination, and continues one to two months postnatally. In the individual lambs of this study the postnatal period of transition ranged between 34 and 48 days. For the blood of the calf, Roos and Romjin[4, 5] found the period of transition from the natal to the adult type of oxygen dissociation curve to be in the neighborhood of three weeks. The oxygen capacity in their calves fell very gradually, rather than sharply, as in the case of some of the lambs; adult oxygen capacities were reached when the individuals were two to three weeks old.

In view of the fact that the most rapid change in the position of the dissociation curve of the blood takes place at the same time as the fall in its oxygen capacity, the possibility must be considered that the change in the position of the dissociation curve is due to the selective removal of the cells containing fetal type of haemoglobin; for Karvonen found in his solubility studies that the transition to the adult type was due to the gradual replacement of fetal type haemoglobin by the adult variety, the proportion of the fetal falling during the transition as that of the adult rose. If the circulating red cells at birth contain but one type of haemoglobin either fetal or adult and not a mixture, then the rapid removal of the cells carrying the fetal type—presumably the older cells—would account for the rapid change in proportions of the fetal and adult types of haemoglobin in the blood and so in the position of the dissociation curve. This possibility is being studied.

Summary

Changes in the character of the oxygen dissociation curve and the oxygen capacity of the blood have been followed during the postnatal period in eight lambs. The oxygen capacity drops toward the adult level within the first fortnight or three weeks after delivery; the dissociation curves shift rapidly toward the right during the first fifteen days after birth and then gradually to reach the adult field between thirty-four to forty-eight days after birth.

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COLORIMETRIC ESTIMATION OF INORGANIC PHOSPHATE AND ACID PHOSPHATASE ACTIVITY IN PLANT TISSUE HOMOGENATES*

KATHERINE S. WILSON AND VICTOR M. CUTTER, JR.

Colorimetric procedures for the determination of phosphate utilizing the stable yellow color which is developed when an excess of molybdate is added to an acidified solution of orthophosphate and vanadate have been described.[2,3] Other workers have developed methods whereby inorganic phosphate and the activity of phosphatases may be estimated in blood serum.[1,4,5] Although cytochemical techniques for the study of the distribution of phosphatases in tissues have been greatly refined in recent years, methods for the quantitative estimation of the activities of these enzymes in plant homogenates or natural fluids are needed.

The method described here was developed during investigations now in progress in this laboratory on the distribution of enzymes in the developing embryo sac of *Cocos nucifera*. These procedures have been extensively used with coconut milk and endosperm homogenates,[6] and preliminary tests indicate that they may be satisfactorily applied to the estimation of inorganic phosphate and acid phosphatase activity in other plant materials. The method measures the amount of residual inorganic phosphate present in homogenates; after incubation of these homogenates with a suitable substrate under controlled conditions, inorganic phosphate is again determined. Phosphatase activity is calculated from the difference between total inorganic phosphate after incubation with substrate and the residual inorganic phosphate of the homogenate.

Materials and Methods

A standard phosphate solution was prepared from reagent grade KH_2PO_4, previously dried to constant weight and dissolved in 7.5 per cent trichloroacetic acid to give a concentration of 100 ug./ml. From this solution a standard series was prepared using 0.1, 0.3, 0.5, 1.0, 1.5, and 2.0 ml., and each tube was made up to a volume of 6 ml. with 7.5 per cent trichloroacetic acid. Two ml. 0.25 per cent ammonium vanadate solution,†

* From the Department of Plant Science, Yale University. This investigation has been aided by a grant from the Jane Coffin Childs Memorial Fund for Medical Research, and in part by a grant-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

† Ammonium vanadate solution was prepared by dissolving 2.5 gm. NH_4VO_3 in 500 ml. boiling distilled water; 350 ml. concentrated HNO_3 were added after slight cooling and the solution diluted with water to one liter.

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1 ml. 0.5 per cent aqueous ammonium molybdate solution and water, to a volume of 10 ml., were added in the order given. After thorough mixing, solutions were allowed to stand at least ten minutes to develop maximum color before reading. Transmission readings were made with a Bausch and Lomb monochromatic colorimeter using the 430u. filter. The instrument was adjusted to 100 per cent transmission with a blank sample prepared precisely as the standards, except that 7.5 per cent trichloroacetic acid was substituted in place of the standard phosphate solution. Per cent transmission of the known KH_2PO_4 standards was plotted on semi-log paper to serve as the standard curve.

Homogenates were prepared by blending known quantities of tissue and distilled water in a Waring blendor for ten minutes or until a smooth homogenate, which could be pipetted with accuracy, was obtained. Homogenates were then diluted when necessary with distilled water to yield galvanometer readings within an appropriate transmission range (20-60 per cent), and 0.5 ml. of coconut milk or endosperm homogenate was pipetted into test tubes each containing 4.5 ml. of 0.5 per cent sodium glycerophosphate solution,* previously warmed to 38° C. The tubes were then incubated at 38° C. in a water bath for two hours, after which they were removed and 1 ml. of 30 per cent trichloroacetic acid was added to stop enzymatic activity. To determine residual phosphate, controls were prepared in a similar manner except that the trichloroacetic acid was added prior to the introduction of the plant homogenate to prevent immediate enzymatic activity, and the tubes were not incubated. As in the preparation of the standard series the reagents were added, to both incubated and control tubes, in the following order: 2 ml. ammonium vanadate solution, 1 ml. ammonium molybdate solution and distilled water to a volume of 10 ml., and the tubes were thoroughly mixed by inversion. Where cloudy solutions resulted they were filtered through Whatman No. 42 filter paper before transmission readings were taken. Inorganic phosphate concentration was determined directly from the standard curve and phosphatase activity was calculated by comparison with the controls.

Summary

A simple colorimetric method for the determination of acid phosphatase activity in homogenates of plant tissue has been described. The method has the following advantages: (i) by simultaneous procedures on the same sample the residual inorganic phosphate content and acid phosphatase

* A stock solution was prepared in distilled water with 1 per cent sodium glycerophosphate (Eastman) and 0.85 per cent sodium diethyl barbiturate. This may be stored at 10° C. under toluene. The substrate solution pH 5, was freshly prepared using 50 ml. of the stock solution, 5 ml. of 1 N acetic acid and distilled water to a volume of 100 ml.

activity are determined; (ii) since the color developed by the test is stable for a period of at least six weeks, the necessity for immediate reading of the results is eliminated and many samples may be tested at one time; (iii) only a short incubation period is required, and determinations can be made on small samples with highly reproducible accuracy.

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IDENTIFICATION OF ACTH IN HUMAN PLACENTAL TISSUE*

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Studies from the laboratory have revealed the presence of ACTH activity in extracts of pregnancy urine which had been heat-treated to destroy gonadotrophic activity.[7] These observations led to the investigation of placental extracts to ascertain whether the source of the ACTH activity was the placenta rather than the pituitary gland. Certain of the results obtained are reported in this paper.

Although the crude methods of extraction employed do not permit quantitative interpretation of yields, the results of this study indicate that significant quantities of ACTH are produced by the placenta. ACTH activity in extracts from placental tissue has recently been described by Tarrantino[8] and in an isolated study by Jailer and Knowlton.[9]

Identification of the type of activity found in the various fractions of placental extracts is based in this present instance upon previous work from this laboratory.[6] Inhibition of the enzyme, hyaluronidase, by adrenal steroids has been shown to be a normal, physiological mechanism and some of the pharmacological effects observed following the administration of extremely large amounts of adrenal hormones may possibly be related to their influence upon this complex enzyme system. Further, ACTH, presumably through its ability to stimulate the release of effective C-11 steroids, was found to be a potent inhibitor of the hyaluronidase-enhanced spreading phenomenon. And finally, a correlation has been obtained between the ability of the C-11 adrenal steroids to inhibit the hyaluronidase-enhanced spreading phenomenon, and *in vitro* inactivation of hyaluronidase as measured by the inhibition of the release of reducing sugars from hyaluronic acid. These concepts have provided in part the basis for the following experiments.

MATERIALS AND METHODS

Placentas were obtained immediately after delivery.† At first, the placentas were extracted in either of two ways—(i) the placenta was directly frozen in dry ice, stored for one day, and then ground and extracted, or (ii) the still warm placental tissue and blood were ground immediately, and further studies were carried out without intervening lapse of time. The crude dry powders obtained from extraction of frozen placentas were found to possess a markedly lower degree of activity, and much lower yields were obtained from them.

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In part, Lyons' procedure[4] for the extraction of mammotropic hormone from pituitary tissue was employed. While based on speculation, it seemed possible that the major portion of ACTH, if present, would be found in the tissue residue rather than in the original acetone-HCl supernate. Hence, the usually discarded acetone residue of tissue and blood was further examined. In determining the properties of the fractional precipitates in terms of activity and toxicity, as a basis for establishing further experimental procedures, the original extractions were made without reference to quantitative yields, pH, or HCl concentration.

The methods for obtaining Fractions I and II were as follows: Fresh, still warm, whole placental tissue and blood (weighing altogether about 1 kg.) were put through a power grinder within a half hour following delivery, and 4 liters of acetone containing 100 ml. of 35% HCl were added to the semi-solid mass; this was mixed thoroughly for two to four hours and then centrifuged. The supernate was decanted, combined with 5 liters of fresh acetone, and agitated (using a mechanical stirrer) overnight in the cold room. The precipitate was separated by centrifugation, redissolved in a small amount of water, and again precipitated with acetone. This reprecipitation was repeated 4 times, with the final dry yield approximately 15 grams. This precipitate was designated Fraction I.

Because of the high water content of the placental tissue and blood, and the likelihood that the major portion of the ACTH activity might remain in the first acetone residue discarded in obtaining Fraction I, further extraction of this residue was made. The tissue residue remaining after the original acetone extraction was suspended in 1 liter of water, mixed well, and stored in the cold room over night. The solid portion was separated by squeezing the mixture through fine cheese cloth. To the liquid fraction, 4 liters of acetone (no HCl) were added. The precipitate which formed was separated by centrifugation, dissolved in water, and reprecipitated with acetone; this procedure was repeated three times. The final product, when acetone-dried, yielded 25 grams, and was designated Fraction II. By reducing the time of the original acetone extraction of the placental tissue to 15-30 minutes, the weight yield of the final dried Fraction II could be increased considerably with no decrease in activity; for example, average yields of 65 grams of Fraction II and 16 grams of Fraction I could be obtained from individual placentas.

The resulting dry powders were dark to light grey in color, and were very water soluble (example, 10 mg./ml.). These preparations were stored in vacuo. The results of other extraction procedures and further purifications of the extracts will be reported in a subsequent paper.

Fresh beef muscle was ground and extracted in the manner as described for obtaining the Fractions I and II from placental tissue. The beef muscle fractions were combined and their effect on the spreading reaction was determined.

Serum albumin* was used as a further control substance; the final dilutions were made from a sterile solution containing 25 mg./ml.

The test substances, placental fractions I and II, beef muscle extract, and serum albumin, were dissolved in physiological saline, and volumes of 0.2 to 1.0 ml. were injected intraperitoneally. The amounts administered varied from 0.5 to 10.0 mg., and the results were determined after varying time intervals of absorption in normal, adrenalectomized, hypophysectomized, and hypophysectomized-adrenalectomized mice. Equal amounts of saline were injected into control groups of mice, and control studies were routinely made. The experimental animals were set up in groups of equal numbers of males and females. There was no difference with mice of different sex, nor did the volume of diluent used exert any effect on the results.

* The vial of sterile serum albumin was kindly supplied by Dr. H. B. Vickery of the Connecticut Agricultural Experiment Station.

In other experiments, to determine whether or not a local inhibitory effect could be obtained, amounts of 0.05 to 5.0 mg. of the fractions of placental extract were injected intradermally in normal and adrenalectomized mice, either simultaneously with, or just prior to, the injection of the hyaluronidase and India-ink mixture. The procedures and methods of measurement employed in the hyaluronidase assays have been described in previously published studies.[5, 6]

Male and female mice of the inbred CBA strain,, 10-12 weeks of age and weighing from 25-30 grams were used. Adrenalectomized mice were tested one or six days after adrenalectomy and were not maintained with extra sodium chloride. Hypophysectomized mice of both sexes were used 48 hours after operation; these mice were fed laboratory chow ad libitum and were given 5% glucose in physiological saline to drink. Examination at autopsy showed the completeness of hypophysectomy and only a slight degree of weight loss and adrenal atrophy.

Since the major inhibitory effects have been found on the hyaluronidase-enhanced areas of spreading, this method of reporting results has been used in most of the studies described in this paper. However, in the testing of placental extracts, control injections of saline and India ink alone were compared with the hyaluronidase-enhanced areas of spreading, thus providing a means of semi-quantitive estimation of the inhibition of exogenously added enzyme (see Table 3). The significance of these determinations has been discussed in another publication.[6]

Standardized preparations of testicular hyaluronidase obtained from Dr. Joseph Seifter of Wyeth Inc. were used. Preparations were designated W-108-A, W-109-A, and W-160-A, and varied markedly in degree of spreading activity.

ACTH preparations designated by Armour as H-8412 were used. The contents of one vial was stated to contain an amount equivalent to 35 mg. LA-1-A and to contain 0.2 unit oxytocin and 0.14 unit pressor substance.

The preparations and potency of the human chorionic gonadotrophin and heat-inactivated chorionic gonadotrophin preparations have been described in an earlier publication.[7]

"Adrin," the Sharp and Dohme commercial brand of epinephrine, was used. Final dilutions were made with saline.

Experimental

Normal animals. Table 1, a summary of representative experiments, shows the influence of crude placental extracts on the spreading reaction.

Inhibition of hyaluronidase-enhanced spreading resulted when mice were injected intraperitoneally with amounts of 1 to 10 mg. of the crude placental fractions I and II. Control groups of mice were injected with saline alone. When 1 mg. of Fraction II is injected, a marked inhibition of hyaluronidase-enhanced spreading was observed with roughly a 60% inhibition of added enzyme. When 5 mg. were injected, essentially maximal inhibition of spreading was observed, while complete inhibition of added enzyme was obtained; 10 mg. of Fraction II caused no greater inhibition and produced no toxic effects. With Fraction I, 1 mg. caused a slight but consistent degree of inhibition of spreading, 5 mg. produced an average of 40 per cent inhibition of spreading (72% inhibition of enzyme), while 10 mg. of Fraction I were necessary to obtain maximal inhibition of spreading and complete inhibition of enzyme. Consistently, Fraction II has proved twice as active as Fraction I.

With doses of less than 0.5 mg. of placental fractions I and II, it was impossible to detect hyaluronidase-inhibiting activity; however, in control studies of inhibition it has been consistently seen that large doses of ACTH must also be used, and amounts of ACTH of less than 0.5 mg. were similarly ineffective. Further, administration of 5 mg. of Fraction II produced

TABLE 1

INFLUENCE OF SYSTEMICALLY ADMINISTERED PLACENTAL EXTRACT FRACTIONS, BEEF MUSCLE EXTRACT, AND SERUM ALBUMIN ON THE DERMAL SPREADING OF INDIA INK WITH HYALURONIDASE*

I. NORMAL ANIMALS

Substance injected	*Dose** Mg.*	*Time between injection and test Hours*	*Time of enzyme spreading Hours*	*Area of spreading*		*% Change from control*	*% Inhibition of added enzyme†*
				Controls Sq. mm.	*Experimental Sq. mm.*		
Fraction I	1	3	3	534(18)	450(12)	—16	~44
Fraction II	1	1	3	535(12)	355(12)	—34	~62
Fraction II	1	1	24	750(12)	510(12)	—32	
Fraction I	5	3	3	534(18)	315(12)	—40	~72
Fraction II	5	3	3	534(18)	252(12)	—53	~98
Fraction I	10	3	3	623(12)	272(12)	—56	~94
Fraction II	10	3	3	623(12)	250(12)	—60	~99
Fraction II	10	3	3	810*(12)	265(12)	—67	~96
Serum Albumin	1	3	3	540(12)	570(12)	+ 5	
Serum Albumin	5	3	3	540(12)	603(12)	+12	
Serum Albumin	10	3	3	540(12)	630(12)	+17	
Tissue Extract	1	3	3	530(12)	592(12)	+ 1	
Tissue Extract	5	3	3	530(12)	578(12)	+ 8	
Tissue Extract	10	3	3	530(12)	590(12)	+11	

* Enzyme with increased spreading activity used. The increased spreading activity had no effect on the subsequently determined maximal degree of inhibition produced, which represented essentially 100% inhibition of added enzyme.

** Intraperitoneal administration. Diluent = 1.0 ml. physiological saline. Control groups were injected with equal volumes of saline alone.

† See Table 3.

the same maximal degree of inhibition of spreading as was obtained with no less than 3.5 mg. of ACTH from the pituitary.

Administration of 1, 5, and 10 mg. amounts of beef muscle extract or serum albumin was completely without inhibitory effect on the hyaluronidase-enhanced areas of spreading.

While factors of non-specificity or toxicity reactions have been essentially ruled out as an explanation for the observed effects following administration of placental fractions, numerous experiments have also shown that toxic doses of colchicine, spermine, formaldehyde, and certain bacterial agents exert little or no effect in producing inhibition of hyaluronidase-

enhanced spreading. Although the placental extract fractions might conceivably provoke a discharge of endogenous epinephrine, it has been demonstrated that the systemic injection of epinephrine in amounts up to 0.02 mg. per mouse produces little or no inhibitory effect. Only when doses of 0.05 mg. or more per mouse were injected was there any degree of inhibition, and this took place only in animals with intact adrenals (unpublished data).

TABLE 2

INFLUENCE OF PLACENTAL EXTRACT FRACTIONS, BEEF MUSCLE EXTRACT, AND SERUM ALBUMIN ON THE DERMAL SPREADING OF INDIA INK WITH HYALURONIDASE

II. ADRENALECTOMIZED ANIMALS

*Substance injected**	*Dose Mg.*	*Time between injection and test Hours*	*Time of enzyme spreading Hours*	*Area of spreading*		*% Change from control*
				Controls Sq. mm.	*Experimental Sq. mm.*	
Fraction I	5	3	1	685(32)	670(6)	−2
Fraction II	5	3	1	685(32)	695(6)	+1
Fraction I	10	3	1	685(32)	850(6)	+19
Fraction II	10	3	1	685(32)	730(6)	+7
Serum albumin	10	3	1	685(32)	678(6)	+1
Beef extract	10	3	1	685(32)	750(6)	+9

* Injected intraperitoneally in volume of 1.0 ml. physiological saline. Control groups were injected with equal volume of saline alone.

NOTE: In intradermal experiments in normal and adrenalectomized animals, these compounds were similarly without inhibitory effect in dosages of 0.05 to 5.0 mg. injected in the intradermal site of hyaluronidase injection, when injections of test substances were made simultaneously with, or just prior to the injection of hyaluronidase and India ink.

The results shown in Table 1 and Figure 1 are representative of several series of experiments in which varying amounts and preparations from different placentas were administrated.

Adrenalectomized animals. Investigations were undertaken to determine the site of action of the placental extract fractions, and whether or not their action might be related to the presence of measurable quantities of the C-11 oxygenated adrenal steroids which are also effective in producing hyaluronidase inhibition.

Groups of male and female mice were tested 24 hours after adrenalectomy. Placental extract fractions I and II dissolved in saline were administered intraperitoneally, and, following arbitrarily selected time intervals of one to six hours, experimental testing with hyaluronidase and India ink solutions was carried out. Table 2 shows that the administration of 1 to 10 mg.

of fractions I or II* was completely without inhibitory effect on the hyaluronidase-enhanced areas of spreading. Serum albumin or beef muscle extract control studies similarly showed no inhibitory effect. In the adrenalectomized animal, cortisone in doses of 0.5 mg. was found effective in producing a marked degree of hyaluronidase inhibition at short post-absorptive time intervals, and Compound F in similar dosages at the longer absorption time intervals was also effective. This would suggest that the activity of these placental extracts was not due to the presence in them of any significant quantities of cortisone or Compound F.

Intradermal experiments in normal and adrenalectomized animals. Intradermal injection of the various placental extract fractions was made in the same site as the injection of hyaluronidase and India ink mixture. The placental fractions were dissolved in saline and combined with the hyaluronidase and India ink solution, or injected just prior to the administration of the enzyme-India ink mixture. The amounts of placental extract injected ranged from 0.05 to 5.0 mg., under the various experimental conditions, and the resulting areas of spreading were measured after time intervals of one to sixteen hours. There was no evidence of inhibition of hyaluronidase-enhanced spreading. Since it has been shown that 0.05 mg. of Compounds E or F, or 0.025 ml. of ACE administered intradermally will markedly inhibit hyaluronidase,[8] the lack of effect produced by large amounts of placental fractions administered intradermally would again indicate the absence of appreciable amounts of the C-11 steroids in the fractions tested.

Hypophysectomized animals. Critical evaluation of the action of these placental extract fractions was obtained by noting their effect on the spreading reaction in hypophysectomized animals. The effects produced by the injection of 1 to 10 mg. of various placental fractions in 48-hour hypophysectomized mice are shown in Table 3 and graphically illustrated in Figure 2. Again, the greater activity of Fraction II is demonstrated. Five mg. of Fraction II or 10 mg. of Fraction I or II produced essentially maximal inhibition of hyaluronidase spreading, with complete inhibition of added enzyme. Inhibition of hyaluronidase did not occur when 1 to 10 mg. of serum albumin or beef muscle extract were administered to groups of hypophysectomized animals.

Potentiation of the hyaluronidase-enhanced spreading appeared to be greater in hypophysectomized animals than was the case with normal animals. It is interesting that hypophysectomized animals better tolerated stress and large doses of these test substances than did adrenalectomized animals under identical experimental conditions. For example, hypo-

* Five and 10 mg. amounts of Fractions I and II were not toxic to the 24-hour adrenalectomized animal; however, the 10 mg. doses proved fatal to the 6-day adrenalectomized animal and in comparison, 5 mg. pituitary ACTH were fatal.

physectomized mice, tested 48 or 96 hours after operation, tolerated without toxic effect doses of 10 mg. of placental extracts, whereas the six-day adrenalectomized animal was killed by 5 mg. ACTH or by 10 mg. of the placental fractions.

A group of hypophysectomized-adrenalectomized animals was given 5 mg. amounts of placental fractions I and II, and hyaluronidase testing

TABLE 3

INFLUENCE OF SYSTEMICALLY ADMINISTERED PLACENTAL EXTRACT FRACTIONS, BEEF MUSCLE EXTRACT, AND SERUM ALBUMIN ON THE DERMAL SPREADING OF INDIA INK WITH HYALURONIDASE

III. HYPOPHYSECTOMIZED ANIMALS

Substance injected	*Dose* Mg.*	*Time between injection and test Hours*	*Time of enzyme spreading Hours*	*Area of spreading*		*% Change from control*	*% Inhibition of added enzyme***
				Controls Sq. mm.	*Experimental Sq. mm.*		
Fraction I	2.5	3	3	655(18)	422(6)	—36	~55
Fraction II	1	3	3	655(18)	392(6)	—40	~65
Serum albumin	1	3	3	655(18)	657(4)	±	
Fraction I	5	3	3	655(18)	302(6)	—54	~86
Fraction II	5	3	3	655(18)	244(6)	—63	~100
Beef Extract	5	3	3	655(18)	670(4)	+ 1	
Fraction I	10	3	3	655(18)	268(6)	—59	~94
Fraction I	10	3	3	650(12)	275(6)	—58	~94
Fraction II	10	3	3	650(12)	270(6)	—58	~94
Serum albumin	10	3	3	650(12)	630(6)	— 3	
Beef Extract	10	3	3	650(12)	680(6)	+ 5	

* Intraperitoneal injection in volume of 1.0 ml. saline. Control groups received equal volumes of saline alone.

** Method of calculation:

Example: Control injections of saline and India ink, average area of spreading (24) = 245 sq. mm.

655 — 245 = 410. Spreading in sq. mm. due to hyaluronidase before testing.
268 — 245 = 23. Spreading in sq. mm. due to hyaluronidase after testing.

Per cent inhibition of added enzyme = 410 — 23 = 387; $\frac{387}{410} \times 100 = 94\%$.

carried out as above described. As in the case of the adrenalectomized animals, no inhibition of hyaluronidase-enhanced spreading was produced.

Although the placental extracts were extremely crude, it is interesting that the maximal degree of inhibition produced with the placental fractions was comparable to that observed with relatively similar amounts of pituitary ACTH. The constant degree of inhibition of spreading in the hypophysectomized animals, with extracts made from different placentas, is to be noted.

Effect on adrenal ascorbic acid. In view of the wide acceptance of adrenal ascorbic acid depletion as an indirect measurement of adrenal activity, the effects of placental extracts on the adrenal ascorbic acid levels of a small number of hypophysectomized rats and mice were determined. In hypophysectomized rats the intravenous injection of 1 and 2 mg./100 gm. of fractions I and II produced an average fall in adrenal ascorbic acid of 20% and 50%, while the injection of 0.1 mg. ACTH was followed by an average fall of 55%. In hypophysectomized mice, 2 mg./100 gm. injected intraperitoneally produced an average fall of 32%. In order to obtain sufficient tissue for analysis, the adrenals of three mice were pooled for each determination of ascorbic acid. With the 2 mg./100 gm. dose, there was an average eosinophil fall of 74% in both hypophysectomized mice and rats.

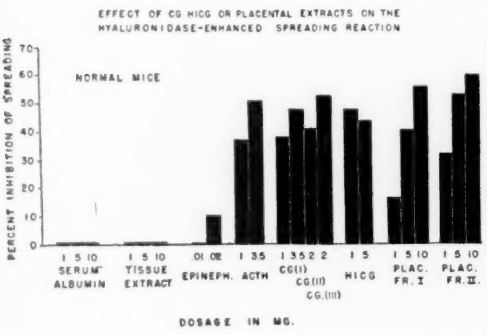

FIG. 1.

DISCUSSION

The markedly similar hyaluronidase-inhibitory effects produced by the various placental extract fractions, by the human chorionic gonadotrophin preparations,[7] compare favorably with the effects produced by the standardized commercial ACTH preparations from the pituitary, in normal, adrenalectomized, and hypophysectomized mice. A graphic summary of some of the results obtained with the use of these preparations in normal animals is seen in Figure 1. Since Fraction II represents material that is normally discarded in the Lyons procedure, it is interesting that Fraction II of the placenta consistently showed a greater degree of activity than did Fraction I, and that more than twice as large a yield of dried powder was obtained in Fraction II. When fresh placental tissue was extracted, the fractions were not only extremely large for the crude procedures employed in extraction, but also possessed ACTH activity in dosage ranges comparable to those for the pituitary preparations. However, placental tissues that had been frozen and stored prior to extraction did not yield fractions comparable in activity or quantity to those obtained with the use of fresh placentas. Thus, a destruction of ACTH activity is indicated, even though the tissue remained in the frozen state. There is indication that this lability of ACTH has been observed in the isolation of ACTH from pituitary tissue by Astwood.[1]

A more critical evaluation of ACTH activity in these placental extracts is seen in Figure 2, which is a graphic summary of results from experi-

ments using hypophysectomized animals. The marked inhibitory effect of the chorionic gonadotrophins and heat-inactivated chorionic gonadotrophin preparations is seen in comparison with results obtained with the crude placental preparations. To indicate further the extent of hyaluronidase inhibition, the results with the placental extract fractions are presented as per cent of actual inhibition of exogenously added hyaluronidase, following the calculation procedure illustrated in Table 3.

The placental tissue fractions caused a marked inhibition of hyaluronidase in both normal and hypophysectomized animals, and with the larger dosages this inhibition approximated 100% inactivation of added enzyme. In all cases the placental extracts were inactive in adrenalectomized animals, and since the inactivation was uniform, this type of experiment was not included in these figures. The possibility that the observed activity was due in any way to the presence of steroids of the nature of cortisone is ruled out by the fact that enzyme inhibition was not observed in adrenalectomized or hypophysectomized-adrenalectomized animals, and by the fact that the placental tissue extracts were without effect when administered intradermally at the site of hyaluronidase injection. Non-specificity was ruled out by the lack of effectiveness of meat extracts and serum albumin, and further supporting this conclusion are the results of numerous studies showing that toxic substances such as formaldehyde, spermine, colchicine, and bacterial agents did not elicit an inhibition of hyaluronidase in the normal or adrenalectomized animal. Epinephrine, in amounts of 0.02 mg. or less per mouse, did not influence the spreading reaction.

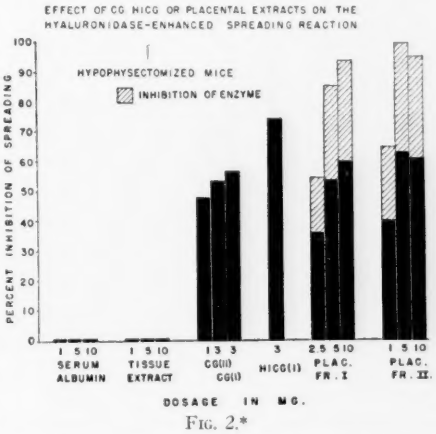

FIG. 2.*

These studies on ACTH activity of placental tissue indicate that the placenta contains relatively large amounts of the hormone. In addition, even though the preparations were extremely crude, there was a striking lack of toxicity with their administration. Since the material is of human

* The more marked inhibitory effect noted with the use of the heat-inactivated chorionic gonadotrophin preparation is due to the fact that a high spreading potency enzyme was employed to test the quantitative aspects of degrees of enzyme inhibition that could be produced. In both types of enzyme testing, essentially 100% inhibition of the exogenously added enzyme was found to have taken place.

origin and presumably would not cause a foreign protein reaction when administered to humans, it is possible that placental ACTH would have an important practical value as there have been recent reports by Feinberg[9] and Thorn[8] of sensitivity to pituitary ACTH preparations. The observation that ACTH activity is obtained with placental extracts is in agreement with the previous report that human pregnancy urine preparations, heated to destroy gonadotrophic activity, possess ACTH activity.[7] These gonadotrophins excreted in the urine during pregnancy have their origin in the placenta,[10] and it is probable that the placental extracts contained the precursor of these excretory products.*

The technique of measuring hyaluronidase inhibition has been developed to the point where it can be applied on a semi-quantitative basis, and has been used as the principal type of evidence for the measurement of ACTH and adrenal steroid activity in this series of studies. However, the pertinent points involved in the establishment of ACTH activity in these placental extract fractions by measurement of hyaluronidase inhibition in the normal, adrenalectomized, or hypophysectomized mouse have been substantiated by studies on adrenal ascorbic acid depletion. In the studies by Tarrantino[6] and by Jailer and Knowlton,[5] depletion of adrenal ascorbic acid has been used as the criterion of ACTH potency. On the basis of these assays, it would appear that extremely large amounts must be administered in order to obtain a significant decrease in adrenal ascorbic acid; however, the gross impurities of the crude extracts must be considered. At the same time, it has been noted that large doses of chorionic gonadotrophins also have to be used in order to obtain adrenal ascorbic acid decreases that are significant. On the other hand, when inhibition of hyaluronidase-enhanced spreading in the hypophysectomized mice is used as a criterion of ACTH activity, the preparations just referred to appear to possess roughly the same order of activity as pituitary ACTH, Armour LA-1-A.

Inasmuch as the experiments herein presented definitely show that there is an adrenal trophic effect, and since epinephrine has been excluded from consideration as a mediator of this action, one must conclude that the effect in all probability is trophic for the adrenal cortex. It is therefore quite appropriate to speak of placental ACTH as a substance which causes the adrenal to produce a factor or factors which inhibit hyaluronidase, but it is not necessary to assume that this placental ACTH has the same physiological characteristics as does pituitary ACTH.

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* Urinary excretion of material possessing ACTH activity is not restricted to pregnancy, since the substance, urogastrone, which possesses many of the chemical properties of chorionic gonadotrophin but which does not have gonadotrophic activity, isolated from normal human male urine, also causes an inhibition of hyaluronidase (unpublished data). It must be remembered that the quantity of material identified as urogastrone in male urine is extremely small, and considerably increases in urine of pregnancy. It is quite possible that the urogastrone isolated from normal male urine originates in the anterior pituitary.

It is obvious that the observations recorded in this paper pose a multitude of questions that must be answered before the relationship between placental and pituitary ACTH can be understood.

Summary

Experimental evidence indicates the presence of ACTH in varying fractions of placental tissue and shows that it is produced in large quantities by this organ. The Lyons' procedure for extraction of ACTH from pituitary tissue is not applicable to the extraction of ACTH from placental tissue and blood.

Administration of various fractions of crude placental extracts to normal or hypophysectomized mice produces a marked inhibition of hyaluronidase-enhanced spreading with essentially complete inhibition of exogenously added enzyme. These fractions are completely without effect in adrenalectomized mice and are without effect when injected intradermally at the site of the hyaluronidase injection.

The inhibitory effect of placental extracts is not due to the presence in them of measurable quantities of steroids of the adrenal cortical type (cortisone or Compound F).

There is a similarity between the inhibitory effects produced by the placental fractions and the results following administration of pituitary ACTH, chorionic gonadotrophins, and heat-inactivated chorionic gonadotrophins.

The presence of ACTH activity in placental extracts is also shown by the depletion of adrenal ascorbic acid and a fall in circulating eosinophils that occurs after their injection into hypophysectomized rats and mice.

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A COLLECTION OF EARLY OBSTETRICAL BOOKS

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Collecting is one of the oldest and most general of hobbies. All sorts of items are collected, from the cheapest kind of articles to rare and valuable art objects and books. I have always been a collector, starting with stamps in childhood, turning to books in maturity, and in recent years, returning to my boyhood liking for stamps, especially those with a medical interest. Beyond question, however, the collecting that has engrossed me most is that of old medical books. I have collected books of outstanding medico-historical interest, and purchased anything that dealt with my own specialty of gynecology and obstetrics. Thus I have assembled thirty-seven obstetrical volumes from the early days of printing, which, I believe, cannot be found in any other single collection anywhere in the world. I have been asked a number of times to describe this collection for the benefit of libraries, collectors, and students, and I have finally undertaken to do so here. I could not have accomplished it without much expert aid. On this score I am deeply indebted to Curt Proskauer and especially to my daughter, C. Doris Hellman, both of whom contributed many productive hours to this task.

I wish to acknowledge the encouragement I derived from the enthusiasm with which the idea of this publication was received by Archibald Malloch, who was until recently Librarian of the New York Academy of Medicine, and from his associates, and from various friends with whom I discussed my plan. Thanks are also due my wife, who helped in the writing.

The most important items in my collection of rare obstetrical books are the 1513 editions of the famous obstetrical textbook, *Der Swangern Frauwen und Hebammen Roszgarten* by Eucharius Roesslin, a physician from Worms. All three were published in the same year but each was set up and printed differently (Nos. 1, 2, 3). One is dated; the other two are not. There is, of course, no way of knowing which of these three was the real first edition, and the surest way of owning the first of this extremely rare and important obstetrical work is to possess all three. Mine are in excellent condition.

Dr. Charles Perry Fisher, formerly Librarian of the Library of the College of Physicians of Philadelphia, wrote me in 1928 concerning the dated (1513) edition, and saying that "Possibly this may be called the first dated edition," and further that "Lang of Rome speaks of a 1508 edition, mistaking the x for a v." The Bibliothèque Nationale catalogue makes this same error.

What is the importance of this rare work, usually referred to as "Rosengarten"? The opinion held until recent times that this was the first printed work dealing exclusively with obstetrical knowledge is erroneous. In 1476 the *Secreta Mulierum* of Albertus Magnus made its appearance in print, and about 1495 the *Buechlein der Schwangeren Frauen,* the so-called "Frauenbuechlein" by Ortolff von Bayerland, also appeared. Both of these can be called obstetrical monographs. The significance of the "Rosengarten" is not due to its being the first printed obstetrical work, but rather to the fact that its text and illustrations resumed a tradition, broken for almost fifteen hundred years. Roesslin's famous pictures of the position of the

FIG. 1. Title-page of the 1513 dated edition of the Rosengarten of Eucharius Roesslin the Elder (No. 1).

FIG. 2. Dedicatory picture appearing on fol. A3*r* of the dated edition of 1513 (No. 1).

Der Frauwen

vnreüwig würt/vnd einer ſchnellen bewegung/von einē ort zů dem andern/die ſie bringt vñ vrſacht zů vnbequēlicher harter geburt. ¶ Zům ſechſtē iſt zů wiſſen/das ein knab vyl ringer zů geberē iſt/dañ ein meytlin. ¶ Zům ſybendē mal iſt die geburt hart vnd ſchwārlich/ſo dz kind zů vyl groß iſt/darumb das es die ſchloß ſeiner mūter nit leichtigēlichē durch tringē mag/Auch wañ das kind zů klein iſt vnd zů leicht/darumb das es ſich vnderſich deſter minder wendet vnd ſenckt/vnd minder mag vß getruckt werden.

¶ Zům achtenmal/darumb dz der kind mer dā eins iſt/oder ein kindt mitt mer gliedern dañ natürlich iſt/beſunder mitt zweyen heuptern/als in diſem.xij.iar/in der graffſchafft Werdēberg/ein kindt mit zweyē heuptern geborē iſt des figur hie bezeuchet iſt/Dz ſo die geburt zů glat iſt/dz ſie dē vßtrucken entwüſt vnnd entſchlüpfft

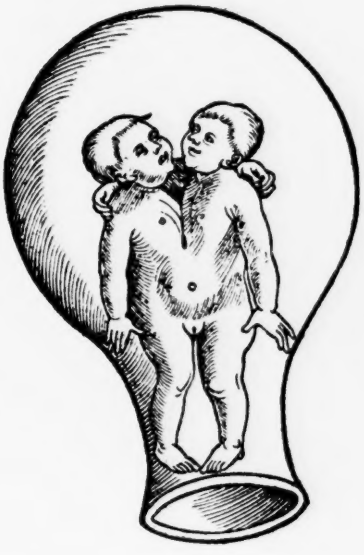
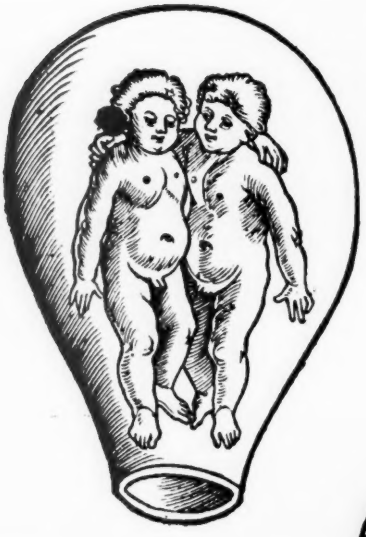


Fig. 3. Two-headed monster on fol. C2v of the 1513 dated edition (No. 1).

Der frawen



¶ Wo aber die zwyling kömen/ mitt den füeſſen/ Sol ſie abermals thün fleiß ankeren/ eins nach dē andern vßfüren/ in maſſen als obſtadt.

¶ So aber der zwyling einer kompt mit dē haupt/ der ander mitt den füeſſē Soll abermals die hebam fleiß ankerē/ dem nechſten zů erſtē helffen/ vnnd dz ander nit vlaſſen/ Dñ das ſoll alſo geſchehen/ on quetzung ir beyder.

Fig. 4. Twins (podalic position and head and podalic position) on fol. E3r of the 1513 dated edition (No. 1).

child closely resemble the sixteen in the manuscripts of antiquity, i.e., Mustio's catechism of women's diseases and midwifery, largely based on Soranus of Ephesus (see Sarton I, 98, 138).

Not only does Roesslin's booklet point back to the distant past, but it also had an enormous influence on the obstetrical practices of the midwives and surgeons of his own time. Latin, the universal language of scholars at that period, was not comprehensible to Roesslin's public. His book in the vulgar tongue or vernacular, the language of the common people, was therefore popular. The great number of reprints of the German edition testifies to this fact, and to the need for such a text for those not conversant with Latin.

The need is further shown by the prodigious number of translations under the most diversified titles: into English by Richard Jonas and Thomas Raynalde (Nos. 19, 20, 21, 22, 23, 24); into French (Nos. 17, 18), the latter by Paul Bienassis; into Spanish, Italian, Dutch (No. 7), Polish, Czech. In addition, especially during the course of the sixteenth and seventeenth centuries and even later, there appeared books which in more or less modified form reproduced his pictures of the position of the child.

Roesslin established the necessity for thorough instruction of midwives. In his versified preface he censured the wretched condition of the current obstetrics, and the ignorance, carelessness, and superstition of the midwives, who brought about unnecessary deaths of numberless newborn. The infant mortality, Roesslin bluntly labelled murder, for which the guilty ones deserved to be buried alive, or "broken on the wheel," instead of being allowed to receive an honorarium for services rendered. In his book, which he wrote at the order of the Duchess Katherine of Brunswick and Lüneburg and which he dedicated to her, he attempted to eliminate, or at least mitigate, these evils.

Eucharius Roesslin, the date of whose birth is uncertain, lived in Freiburg, in Breisgau, in the last decade of the fifteenth and the beginning of the sixteenth century. In 1506 he left there and went to Frankfort on the Main. He remained at Frankfort until 1511 with only a slight intermission in 1508 when he was at the court of the Duchess Katherine. In 1513 he became town physician in Worms. He returned to Frankfort in the same capacity in 1517. There he continued active at his post until his death in 1526.

His son, also named Eucharius, who had studied in Cologne, Freiburg, and Leipzig and was a licentiate in medicine, succeeded him as town physician in Frankfort on the Main. The similarity of name and professional employment caused them to be confused with each other in the literature, or to be held as one and the same person.

Eucharius Roesslin, the Elder, wrote *only* the "Rosengarten." After his death it was brought out by his son under the title of *Ehstands Artzneibûch*

(Nos. 9, 10) with the appended *Heymlicheyten* by Albertus Magnus, *Sorgliche zůfellen der schwangeren Frawen* by Ludovicus Bonatiolus, *Frauen Artzney* by John Cuba, and *Kindspflegung* by Bartholomaeus Merlinger. After the death of the younger Roesslin in 1553, it was published by the town physician of Frankfort, Adam Lonicer, as *Hebammenbuechlein.*

Following his father's demise, the younger Roesslin published a Latin translation of the "Rosengarten" under the title *De partu hominus* (Nos. 11, 12, 13, 14, 15, 16). In accordance with the contemporary custom among scholars, he used the Greek form of his name and styled himself "Rhodion" (Roschen, or Little Rose) on the title-page. It was the Latin edition that was largely used for translation into French, English, Dutch, Italian, and other languages.

The younger Roesslin also brought up to date and reissued, with many woodcuts, the *Ortus Sanitatus,* allegedly by Johann de Cuba (John Cuba), at one time town physician in Frankfort. This aroused great interest during the sixteenth century. The younger Roesslin's edition first appeared in 1532.

Following custom, Roesslin treated botany as a subdivision of medicine and not as a separate science. His voluminous botanical textbook rapidly found favor with physicians, apothecaries, gatherers of herbs, distillers, and mountebanks, so that it passed through a large number of editions. It remained the chief authority in its field for a long time, with the result that only a few copies have come down to us, most having been worn out in use.

In the "Rosengarten" the Senior Eucharius Roesslin spoke not only from his own obstetrical experience, but quoted passages from the best known medical authors of antiquity and the middle ages, such as Hippocrates, Galen, Rhazes, Avicenna, and Albertus Magnus. Distinguishing his work from the works of his predecessors are the gynecological and obstetrical descriptions which he added, and, above all, the seventeen little pictures of the different positions of the foetus *in utero.* These are included in all the editions of the "Rosengarten" and its variously titled later editions, and in the several translations.

The representation of twins is new; the remaining sixteen constitute the same number as in the several illustrated manuscripts. As we know today, Mustio's illustrations can be traced back to Soranus of Ephesus, the great gynecologist and obstetrician who lived in Rome at the time of Trajan and Hadrian. Either Soranus was translated by Mustio, or Mustio's work was based on the work of the former. Soranus described the various faulty positions of the child in detail, deeming them important because of their significance in labor and delivery.

In Roesslin's presentation of the foetus *in utero* we see the same bottle or balloon, resembling a more modern cupping-glass, to which Soranus and Mustio had previously compared the womb (see both the famous Mustio

manuscripts, that of the twelfth century in Copenhagen and that of the thirteenth in the Vatican). Since this codex, which is now to be found in the Vatican, was in the library of the Castle at Heidelberg until the year 1623, it is probable that Roesslin saw it there while on a visit from nearby Frankfort or Worms, and that he copied its pictures in order to use them as illustrations for his "Rosengarten."

From the practical obstetrical standpoint, the significance of the "Rosengarten" lies also in the fact that Roesslin again brought to the fore the knowledge of podalic version which had been almost forgotten since the time of Soranus and Mustio. He thus limited cephalic version, which has more theoretical merit but is less practical in execution.

The normal position of the foetus, according to Roesslin, is the head (cephalic) presentation with the hands on the upper thighs, a view which Soranus had already presented. As the next most favorable position, Roesslin designates the complete footling presentation, provided that the arms lie against the body and the hands touch the upper thighs. The same footling presentation, but with the arms directed upwards, "So das Kind erscheinet mit beiden Fuessen und hat die Hend nit neben ihm unter sich gestrecket, sondern über sich," is represented as the most dangerous. Strangely enough Roesslin does not consider the transverse position in any way hazardous.

The three membranes surrounding the foetus he calls the "Bueschlein," or "Nachgeburt," "Biles" and "Armatura Conceptis." He describes the signs of labor as pains in the back, pains in the abdomen, pains in the genitals, and heat in the uterus. In dealing with the period of pregnancy, he recommends a laxative diet for healthy women and a strengthening diet for weak ones. He mentions the great survival power of the seven months' child, ascribing to it a greater vitality than that possessed by the child carried *in utero* for eight months. Today this is, of course, considered erroneous. He also attributes difficult delivery to the smallness of the uterus, to stenosis of the cervix as a consequence of pathological changes in it, abdominal tumors, hemorrhoids, asthenia of abdominal pressure, depressed morale (the psychosomatic designation of that day), abnormal largeness or smallness of the child, pregnancy with twins, too early ending of the pregnancy, too light or too heavy membranes, rupture of the membranes, and death of the baby.

Roesslin recommends a half-sitting position for the woman in labor, preferably on a special birth-stool, whose representation can be found in most editions of the "Rosengarten" and its translations, and which Soranus had already described fourteen hundred years earlier. The stool is here described from a German translation of Soranus:

> In der Mitte des Stuhles und dort, wo die genitalien ruhen, soll ein kleine halbmondfoermiger Raum ausgeschnitten sein. Dieser darf nicht zu gross, damit die Frau nicht bis zu den Hueften einsinke, noch zu klein

sein, da sonst die weiblichen Scham gepresst wird, was noch laestiger ist. Denn eine Hoehlung die zu weit ist, kann man mit Leinwand zustopfen, Die Stuhlplatte sei derartig, dass auch recht starke Weiber darin Platz haben, die Hoehe im Verhaeltnis dazu. Kleineren Personen kann eine Fussbank nachhelfen. Der unter dem Sitze befindliche Teil des Stuhles soll an den Seiten mit Brettern beschlagen, vorne und hinten dagegen offen sein zur Verwendung beim Geburtsakt. Ueber dem Sitze sollen an beiden Seiten zwei Querhoelzer . . . eingefuegt sein, damit man in diesen bei den Anstrengungen die Haende der Gebaerenden stuetzen kann, im Ruecken befinde sich eine Lehne, welche den Hueften und dem Becken das Zurueckweichen unmoeglich mache.

In some editions of the "Rosengarten" the birth-stool is portrayed with drapery of material instead of the boards on the lower side. Jacob Rueff, of Zürich (Nos. 29, 30, 31), expressly required this. He says the birth-stool should have four legs or feet, covered with black material so that the child will not be injured, and so that, if needed, the women helping the midwife can get their hands inside the space protected for the actual delivery, and for the baby.

Soranus gave exact instructions for the type of aid to be rendered by the midwife and her assistant during labor. These regulations were repeated by Roesslin. The midwife in clean clothes sits opposite the laboring woman, but a little lower—because the arrival of the foetus ("frucht") proceeds from above downward. Roesslin recommends one or two helpers instead of the three helpers recommended by Soranus. They are pictorially presented in the woodcuts of the "Rosengarten" and the *De Conceptu et Generatione Hominis*, by Jacob Rueff, town surgeon of Zürich.

The last chapters of the book of Roesslin the younger deal in detailed manner with the care of the child from birth until after weaning. They discuss the various diseases of the nursing child. At the close of the "Rosengarten" is an alphabetically arranged appendix of the Latin words appearing in the book, and their German translation.

An English edition based on the Latin "Rosengarten" was published by Richard Jonas in 1540. (Item 19; also in Huntington Library.) This bears the title *The byrth of Mankynde, newly translated out of the Laten into Englysshe*. All that is known about Jonas is that he was a "studious and diligent clarke." His edition is dedicated to the "Most gracious, and in all goodnesse most excellent vertuous Lady Quene Katheryne (Howard), wyfe and most derely belovyd spouse unto the moste myghty sapient Christen prynce, Kynge Henry the VIII," just as Eucharius Roesslin dedicated his "Rosengarten" to the Duchess of Braunschweig and Lüneburg.

The next English edition appearing in 1545 was no longer by Richard Jonas but by Thomas Raynalde "Phisition." In this the contents were "newly set furth, corrected and augmented," and formed the basis of the many later English editions credited to Thomas Reynalde, "Doctor of Physick." (In some editions he is called Raynald or Raynalde.) Contrary

to Richard Jonas who had produced a literal translation, Raynald gave a free translation with voluminous original additions.

The "Byrth Fygures" with the "Woman's Stool" are taken from the Roesslin *De Partu Hominis*. Whereas in all previous editions the pictures of the position of the child were woodcuts, they now appeared for the first time as engravings. These are to be counted among the earliest English engravings. (They are probably the very first.)

In England there was urgent need for these popular editions of an obstetrical textbook in the language of the country just as earlier a similar need existed in Germany. Neither the midwives nor most of the surgeons were versed in Latin, nor any other foreign tongue; consequently, they could learn only from textbooks in their own language. Thomas Geminus brings this out plainly in the preface of his Vesalius Compendium (1553), an anatomy translated into English ". . . by the informacion of sondrye frendes and also of diuerse surgeons I haue ben borne in hande, that the same worke beeyng set foorth in the Englishe tounge might greatly auaile to y^e^ knowlage of the unlatined Surgeons, & by meane of them, should bee muche more beneficiall, then in latin it is to an infinite nombre of people in thys your Maiesties Royalme of Englande. Wherefore mynding to rendre vnto this Countrey, . . . as muche fruite as my poore industrie and continuall trauaill maye possibly bee hable to yelde, I haue earnestly applyed my selfe to make common and familiar to all Englishe people, that which in the latin hath ben found profitable amonge so many foren nacions. . . ."

The great number of English editions which were subsequently issued attest the timeliness of Jonas's and, later, Thomas Raynalde's decision to translate the *De Partu Hominis* into the common tongue (vulgar tongue). By way of his son's Latin translation, the Jonas literal version, and finally, Raynalde's revision, Roesslin's "Rosengarten" exercised a deep influence over the practice of obstetrics in England for a long time.

Raynalde's preface to the *Byrth of Mankynde* is entirely independent of Roesslin. It is especially interesting because it depicts the origin of his book, *The Woman's Booke*, and mentions the resistance and opposition which he met in connection with it. Criticism hinged principally on the fact that such "delikate" matters as the anatomy of the female generative organs were openly discussed and even pictorially presented "forsomuch as therein was descried and set foorth the secrets and priuities of women and that every boye and knave had of these bookes, reading them as openly as the tales of Robin hood and etc."

These pictures of "all the inward parts of women," these "lively and expresse figures" are not to be found in Roesslin's "Rosengarten" nor in the early editions of the *De Partu Hominis* of his son, so that the reader is likely to conclude that they were especially drawn for Raynalde's work, and are thus the first published anatomical drawings. But this is not the

case. These illustrations are with "shameful supression of their origin," taken from Vesalius' *De Humani Corporis Fabrica* which appeared in 1543. Even the descriptive text of the pictures of Raynalde is borrowed word for word from Vesalius's classic anatomy.

Just as Raynalde adopted the text and pictures of other authors (Roesslin and Vesalius) without naming them—a practice which at that time was not considered dishonorable—so did later writers. The younger Roesslin in his later editions of *De Partu Hominis* added pictures of the womb and placenta from Vesalius's Anatomy without giving the source.

New pictorial material by other authors and publishers was furnished for the first time in the 1554 edition of the *De Partu Hominis*. This consisted of amusing little pictures from the life of the nursing infant: its care and upbringing, the infant's bath, its appearance in swaddling clothes, and its learning to walk in a moving stand.

One of the most notorious plagiarists was the Strassburg surgeon, Walter Hermann Ryff. He robbed the works of others, completely without scruple. Vesalius wrote of Ryff in a letter to a learned friend, the printer, Johan Oporinus:

> Argentinensis ille—de studiis pessime est meritus, quod tabulas, quae nunquam satis magnae studiosis proponi poterunt, tam foede contraxerit et turpissime pictas ac praeter omnem rationem circumscriptas cum Augustani versione tanquam suas emiserit.
>
> The fellow at Strassburg played a scurvy trick on scholars in that he so shamefully cramped the engravings which could not have been too large for the best results to scholars, and issued them as his own, wretchedly reproduced, and reduced in size out of all reason, with the text of the Augsburg version. (Choulant, 186)

In his book *Des Aller fürtrefflichsten Geschöpfs* (Nos. 27, 28), Ryff used not only Roesslin's pictures of the position of the child, but also the anatomical representations of Berengario, Dryander, Ketham, and Vesalius. In Ryff's *Frauen Rosengarten* which appeared during the life of the younger Roesslin, he shamelessly imitated and abbreviated, and even appropriated, some of the same titles. Ludwig Choulant said of Ryff, underlining the opinion of the great Swiss Gesner, that Ryff lived "bei einem unstetten Aufenthalt ohne viel eigenes Wissen von Zusammenschreiben ärztlicher Compendien für sehr verschiedene Zweige dieser Wissenshaft und war in Allem ein unverschämter Plagiarius."

Walter Ryff not only copied the Roesslin "Rosengarten," but what was even more unfortunate he added antiquated theories and prescriptions which he found in earlier medical works. His bungling volume is in striking contrast to the fine obstetrical book of the Zürich "Lithotomist, town surgeon, obstetrician and poet playwright," Jacob Rueff. Rueff was described by his professional colleague and friend, Conrad Gesner, "Vir in sua arte

peritissimus et mihi amicus" (A man skilled in his art, and my friend). Rueff's writings revealed a measure of independence of his predecessors and a respect for the newly acquired anatomical knowledge, although his *Schön lustig Trostbüchle von den empfengknussen und Geburten der Menschen* (the Latin edition of the same date has the title *De Conceptu et Generatione Hominis*) included pictures of the position of the child scarcely differing from those of Roesslin. He corrected some representations which obviously appeared too fantastic. Reflecting the influence of Vesalius, he added illustrations of coarse-grained ovaries and depicted the urethra as emptying in the middle of the vagina.

Through his position as town surgeon and teacher of obstetrics in Zürich, Jacob Rueff naturally had broad practical experience in gynecology and obstetrics. This is reflected in his compilation which introduces new obstetrical instruments, especially the toothed duck-beak for the extraction of the dead child, and other forceps after the patterns of the Arabs. Jacob Rueff advocates birth in foot presentations with certain qualifications, recommending the exercise of pressure from the outside on the after-coming head. For cephalic version he recommends a method "Schybung des Kinds," which is probably the first example of version by combined internal and external manipulation. Rueff repeats Roesslin's pictures of the birth-room and the lying-in room which we have already described in the "Rosengarten" but he altered many of the pictures of the position of the foetus.

Walter Ryff plagiarized the Vesalian anatomical representations. It is to the credit of Jacob Rueff that when he copied the Vesalian pictures he acknowledged their origin. Rueff was convinced of the importance to midwives of anatomical knowledge of the internal female organs, and drew attention to the Vesalian illustrations. He was the first to use true anatomical pictures in an obstetrical book. His female figure will be found in an illustrated plate with a description. An oval is cut out of the anterior wall of the "bärmutter" (uterus) so that one can look into it and see the foetus. The prepared generative organs, essentially clear and easily surveyed, are repeated in still another illustration. In the last section the book shows thirty-one figures portraying the most varied and fantastic abnormalities ("Monstra," "Ostenta," and "Portenta").

Roesslin's pictures of the position of the child which we first saw in the 1513 edition recur a century later in the English edition of *Childbirth* by Paré's student, Jacques Guillemeau (Nos. 35, 36). They are presented with only slight alterations and additions. The picture of the umbilical cord is the most conspicuous of the changes.

Even this is not the last time that Roesslin's foetus *in utero* appeared in medical works. To describe or even mention those which are not part of my collection would lead too far afield and would not fit into the framework of this catalogue.

SHORT TITLE LIST OF ROESSLIN'S "ROSENGARTEN"*

No.	*Title*	*Date*	*Place of publication*	*Publisher*
1.	Rosengarten	1513	Strasburg	Martin Flach Jr.
2.	Rosengarten	[1513]	[Hagenau]	[Gran] woodcut on Title
3.	Rosengarten	[1513]	[Hagenau]	[Gran] ornamental border on Title
3a.	Rosengarten	1528	Augspurg	Heinrich Steiner
4.	Rosengarten	1529	Augspurg	Heinrich Steiner
5.	Rosengarten	1529	Strasburg	Balthassar Beck
6.	Rosengarten	1537	Augspurg	Heinrich Steiner
7.	Rosengarten	1540?	Antwerp	Jan van Ghelen
8.	Rosengarten	1910	Munich	Carl Kuhn
9.	Ehestandsarzneibuch	1533	Frankfort	Egenolph
10.	Ehestandsarzneibuch	1565	Frankfort	heirs of Raben and Weygand
11.	De partu hominis	1532	Frankfort	Egenolph
12.	De partu hominis	1536	Venice	Bindonus
13.	De partu hominis	1544	Frankfort	Egenolph
14.	De partu hominis	1551	Frankfort	Egenolph
15.	De partu hominis	1556	Frankfort	heirs of Egenolph
16.	De partu hominis	1563	Frankfort	heirs of Egenolph
17.	Des divers travaux	1539	Paris	Foucher
18.	Des divers travaux	1577	Paris	Bonfons
19.	Birth of Mankind	1540	London	T[homas] R[aynalde]
20.	Birth of Mankind	1552	London	Tho[mas] Ray[nalde]
21.	Birth of Mankind	1565	London	Richard Jugge
22.	Birth of Mankind	1604	London	Thomas Adams
23.	Birth of Mankind	1613	London	Thomas Adams
24.	Birth of Mankind	1634	London	A.H. (sold by Morret)
25.	Kreuterbuch	1536	Frankfort	Egenolph
26.	Kreuterbuch	1546	Frankfort	Egenolph
27.	Anatomy (Walter Hermann Ryff)	1541	Strasburg	Beck
28.	Rosengarten (Walter Hermann Ryff)	1545	Frankfort	Egenolph
29.	De conceptu (Jacob Rueff)	1554	Zurich	Froscheverus
30.	De conceptu (Jacob Rueff)	1580	Frankfort	Corvinus, Feyerabend
31.	De conceptu (Jacob Rueff)	1587	Frankfort	P. Fabricius, Feyerabend
32.	Hebammen Buch (Jacob Rueff)	1588	Frankfort	[Feyerabend]
33.	Von Weybarn vnd Geburten der Kinder (Albertus Magnus)	1579	Augspurg	Manger
34.	De generatione et partu (Terelius)	1578	Lyons	Marsilius
35.	Childbirth (Guillemeau)	1612	London	A. Hatfield
36.	Childbirth (Guillemeau)	1635	London	Anne Griffin for Norton and Whitaker

* A complete description with additional illustrations will be published with the reprints.

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ACUTE RENAL FAILURE

Lower Nephron Nephrosis*

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The term lower nephron nephrosis[30] is a recent embellishment in terminology for a pathological process that has a venerable and distinguished history. This syndrome may result from a variety of circumstances. It can arise from the use of sulfonamides, carbon tetrachloride, mercury, or numerous other drugs or poisons; from injuries which cause massive destruction of tissue, such as crushing injuries and burns; and from the destruction of blood cells within the circulation as the result of drugs, disease (e.g. blackwater fever), or transfusion with incompatible blood. Prolonged peripheral circulatory collapse may have a similar effect.[18, 24, 37, 43, 44]

Gross examination of the kidneys may reveal nothing more than slight enlargement and a pale swollen cortex. Histological examination usually reveals the presence of pigment and hyaline casts in the lower parts of the nephrons. There may be necrosis of the epithelium of any part of the tubule. Although in some instances the necrosis is most severe in the proximal tubular cells, the injury is most constant and usually most severe in the lower segments of the nephrons. Aside from mild swelling of the cells of Bowman's capsule early in the process, there are no discernible lesions in the glomeruli.[20, 21] Since the necrosis is almost entirely confined to the epithelial tissue, the process is reparable. Evidence of regeneration of the tubular epithelium may be seen as early as the fourth or fifth day of the disease. In two weeks the epithelium is largely restored and functioning, and in several months it may be impossible to detect any signs of the injury.[4] The most important inference to be drawn from the nature of the pathological process is that should the patient survive his other injuries, the renal lesions will be repaired.

The clinical expression of this pathological process is severe renal insufficiency with oliguria or anuria. The mechanism responsible for this has been disputed.[1] Some have claimed that the tubules are mechanically blocked by pigment casts and crystalline precipitates, solid or viscous liquid in character.[13] This seems unlikely as a major factor, since it is rare to find casts in more than twenty per cent of the tubules.[24] Moreover, dilatation of the tubules proximal to the casts is not a consistent phenomenon and seems dependent upon the manner in which the tissue is fixed prior to preparation of histologic sections; the dilatation of tubules may be largely an artefact.[21] Richards,[35] by direct microscopic observation of the kidneys of frogs

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poisoned with bichloride of mercury, noted that the power of active reabsorption and selective retention of diffusible substances by the renal tubules was abolished. Moreover, despite anuria, there was at least a normal rate of formation of glomerular filtrate. The implication of these observations is that the necrotic tubular epithelium was no longer a discriminating membrane, and that the bulk of the glomerular filtrate passed back into the body fluids through the diseased renal tubular epithelium. Less direct evidence of "back diffusion" is available. The U/P ratios of two substances that are excreted by the process of filtration only, without secretion or reabsorption by the tubules, should be identical. Simultaneous examination of the U/P ratios of creatinine and inulin in the dog has shown that these two ratios are not identical in lower nephron nephrosis.[37] Negative values for Tm_{PAH} which imply reabsorption (back-diffusion) of PAH at some point in the renal tubule have also been described in this disorder.[32] Since the tubular cells do not constitute a discriminating membrane in lower nephron nephrosis, the current techniques used to measure the rate of glomerular filtration and renal plasma flow may be invalid and cannot be interpreted with confidence in this disorder.

The pathogenesis of lower nephron nephrosis appears to be largely dependent on two factors: (i) some substance that has the ability to destroy renal tubular epithelium, abetted by (ii) anoxia of the renal tissue. These factors and the site of the most marked lesions of lower nephron nephrosis have physiological implications that are relevant to its prevention and treatment.

The modern concept of the formation of bladder urine is that a large volume of glomerular filtrate is converted to a much smaller volume of fluid which may vary considerably in composition and tonicity. The urine that reaches the distal tubule is reduced to about a fifth of its original volume, the composition has been altered, but it is still isotonic with the glomerular filtrate. It is in the distal tubule (the lower nephron) that water and solutes may be differentially reabsorbed, producing a hypotonic, isotonic, or hypertonic bladder urine.[28, 30, 47]

The extent to which the water is reabsorbed in the distal tubule is largely dependent upon the action of the antidiuretic hormone of the posterior pituitary gland. Verney's experiments[45] indicate that the secretion of antidiuretic hormone by the posterior pituitary gland is automatically regulated in accordance with the tonicity of the extracellular fluid. He postulates the existence of osmoreceptors somewhere in the region of distribution of the internal carotid artery which respond to variations in the effective osmotic pressure of the fluid about them. An increase in the tonicity of the fluid stimulates this receptor which, in turn, transmits the stimulus through the supra-optico-hypophysial pathway ultimately to promote the secretion of posterior pituitary hormone. Similarly, a decrease in the tonicity of the fluid bathing this receptor leads to a reduced stimulus to the posterior

pituitary gland and a graded suppression of secretion of antidiuretic hormone.

Certain lines of investigation strongly suggest that the neurohypophysis (directly or indirectly), or some other antidiuretic mechanism, may respond to stimuli other than those related to tonicity. Brun, Knudson, and Raaschou[2] demonstrated that the decrease in urine flow produced by the stationary standing position, while sustained in normal persons, was quite transient in patients with diabetes insipidus. Judson, Epstein, *et al.*,[17] observed the same difference in response between normal human subjects and patients with diabetes insipidus who were subjected to experimental venous congestion of the limbs. An antidiuretic response is often associated with a contracting plasma volume following a large paracentesis in cirrhotics with ascites.[20, 26] Holmes and Cizek[15] observed an increase in thirst with some retention of water in excess of salt in dogs whose extracellular fluid and plasma volumes had been diminished by experimentally induced salt depletion. According to Leaf and Mamby[19] the serum of dogs with contracted extracellular volume and hyponatremia has more than the usual antidiuretic activity.

These data support the hypotheses that an increase in tonicity of the body fluids and a contraction of the extracellular volume may both be stimuli for release of antidiuretic hormone, which increases reabsorption of water in the distal tubule and concentrates the solutes present in the distal tubular urine.

It is logical to assume that if there is a noxious agent among the solutes of the distal tubular urine, an increase in its concentration will promote a greater degree of damage to the tubular cells. There is direct experimental and clinical evidence to support this. Haskell, *et al.*,[14] found that eighty per cent of dogs survived a usually lethal dose of bichloride of mercury if a diuresis was promoted by an injection of normal saline soon after the administration of this poison by stomach tube. Corcoran and Page,[5] Harrison, *et al.*,[13] and Maluf[22] report that it is more difficult to produce renal insufficiency with intravenously administered hemoglobin in well-hydrated animals. Peters, *et al.*,[20] proposed a radical but successful departure from the conventional mode of early therapy in patients suffering with intoxication from bichloride of mercury that emphasized the promotion of an adequate urine flow by the prompt administration of fluids. Further insight may be gathered with respect to this problem from a consideration of the fact that a hemolytic transfusion reaction rarely leads to lower nephron nephrosis in patients with renal insufficiency characterized by an inability to concentrate urine.

The modifying influences of the state of hydration, circulatory dynamics, and the concentration of the urine on the development of lower nephron nephrosis are strikingly illustrated in the responses of a patient (C23592) to three separate hemolytic reactions. The first of these occurred during

convalescence from a right thoracolumbar sympathectomy. The patient was not dehydrated and was given an infusion of a liter of normal saline following the reaction. It is reasonable to assume that this was a hemolytic reaction, since the urine became dark in color and gave a positive reaction to guaiac, and there was an elevated concentration of total bilirubin in the serum. Despite this, there was no suppression of urine flow. A second hemolytic transfusion reaction occurred on the day of the second operation. This reaction was superimposed on a background of dehydration and peripheral vascular collapse and was followed by a lower nephron nephrosis. She sustained a third hemolytic reaction to an infusion of washed red cells on the fifteenth day following the onset of the lower nephron nephrosis. This reaction occurred at the time when the patient was in the diuretic phase of recovery from the prior renal tubular damage. The diuresis of dilute urine continued without interruption. The absence of a highly concentrated urine associated with the normal state of hydration and the infusion of saline at the time of the first reaction, and the inability to concentrate the urine at the time of the last hemolytic reaction are probably the factors responsible for the lack of injury to the renal tubules. In contrast, the development of lower nephron nephrosis following the second reaction was probably conditioned by the state of dehydration and peripheral vascular collapse.

It has been claimed that an acid reaction in the urine enhances the deleterious effect of a noxious agent on the tubular epithelium. However, several groups of investigators[5, 9, 13, 22] have established that the increased damage is associated with the dehydration that may accompany the acidosis and not with the acidosis *per se*. When the urine of experimental animals was made acid, but care was taken to assure proper hydration, there was no increase in the incidence of renal insufficiency following the administration of solutions of hemoglobin, methemoglobin, or hemolyzed erythrocytes. The beneficial effects of infusions of alkaline solutions in hemorrhagic shock are not striking[16] and may be related to the effects on volume and tonicity of body fluids rather than to acid-base balance.

A contracted extracellular fluid volume may influence the development of lower nephron nephrosis by a mechanism unrelated to its influence on the concentration of urine in the distal tubule. A decrease in the volume of the extracellular compartment resulting from dehydration is usually apportioned between the two major components of this compartment, the interstitial fluid and the plasma. The degree to which blood volume loss may be compensated by a movement of fluid from the interstitial space into the blood stream is conditioned, in part, by the volume of the interstitial fluid. Warren, Merrill, and Stead[18] found that in dogs subjected to tourniquet shock the quantity and pressure of the extracellular fluid was as important in determining the size of the plasma volume as was the quantity of circulating plasma protein. Weston, *et al.*,[18] investigated the response to hemor-

rhage in dogs whose intake of water had been restricted for eighteen to thirty hours prior to the bleeding, as compared with a group which had been allowed free access to water. It required a smaller bleeding to produce shock in the dehydrated than in the control animals. Others[16] have reported that the administration of normal saline prior to experimental hemorrhage protects animals against an otherwise lethal bleeding. Moreover, a contracted plasma volume associated with dehydration, even in the absence of hemorrhage, may be sufficient in itself to lead to a compensatory selective vasoconstriction in certain organs in the interest of maintaining an adequate flow of blood to more sensitive areas. The renal vascular tree participates in this vasoconstriction[18, 43, 49] and the renal blood flow is often reduced. Since this vasoconstriction aids in the maintenance of a normal blood pressure despite a decrease in the blood volume, renal ischemia and consequent anoxia may precede gross evidence of peripheral vascular collapse. This anoxia may in itself produce severe acute renal insufficiency,[12, 31] and it potentiates the effect of other nephrotoxic agents. Lastly, the prolonged vasoconstriction may eventuate in irreversible peripheral vascular collapse.[10, 11, 33, 34, 50]

The preparation of patients for scheduled surgical procedures frequently provides for the omission of all food and fluids for twelve or more hours prior to operation. Losses of fluid are incurred during this period by way of the urine, insensible perspiration, and sweat. The latter will vary in amount depending on fever and the weather. The patient at the time of operation is in the so-called hydropenic state, in which it is postulated that there is near-maximal antidiuretic activity with a urine flow of about a half cubic centimeter a minute. In the operating room the patient, covered with several layers of cloth, sustains additional losses of fluid as insensible perspiration, sweat, and frequently vomitus and blood. It has been estimated that in the course of a major operation under general anesthesia in average climatic conditions, a patient may lose at operation two to four liters of fluid through the skin and respiratory passages. Losses may be even greater during prolonged operations in extremely hot weather. General anesthesia in itself has an antidiuretic effect. Thus, the two stimuli calling forth antidiuretic activity, increase in tonicity, and contraction of the body fluids, co-exist. The fluid losses, in themselves, may be sufficient to promote a state of peripheral vascular collapse, for which the most common therapy is transfusion of whole blood. If the operation is a long and difficult procedure, the losses of fluid will increase and many transfusions may be administered. Each transfusion carries with it the hazard of a reaction which may be hemolytic in character. Should such an unfortunate accident occur, it has been allowed to develop at a time when the products of hemolysis will be maximally concentrated in the distal tubules, and when these tubules may have endured anoxia secondary to renal vasoconstriction. The stage is admirably prepared for the development of a lower nephron nephrosis.

Insofar as proper hydration can modify this unfavorable setting, it should likewise prevent or at least minimize this type of lower nephron nephrosis. Proper hydration does not imply that the patient need be given inordinate volumes of fluids, but enough saline and glucose solution should be administered at the start of an operation to compensate for antecedent losses and to provide for the losses of salt and water that may be anticipated during the operative procedure. Continuous intravenous infusion of five per cent glucose in water and some saline, regulated to promote the excretion of a reasonably dilute urine and to compensate for losses of sweat and blood, will provide protection against the concentration in the distal tubule of a substance toxic to the renal tubular epithelium. Since dehydration itself contributes to the production of shock, it is quite possible that the provision of these fluids in advance may reduce the necessity for transfusions.

This discussion should in no way be interpreted as a condemnation of the use of transfusions. The availability of whole blood and the ease with which it may be administered represent a major advance in the therapy of shock. Certain considerations, however, are implicit in this argument: (i) peripheral vascular collapse may be primarily caused by, or certainly contributed to, by losses of fluids other than blood; (ii) to the extent that dehydration can cause or contribute to peripheral vascular collapse, this latter can be prevented or modified; therefore, proper hydration will reduce the necessity for transfusions; and (iii) since the use of multiple transfusions increases the risk of a hemolytic reaction, there is a specific responsibility to prepare the patient in such a manner that this type of accident will lead to as little damage as possible.

The management of a patient before and during a surgical procedure has been selected simply for the purpose of illustrating the nature of the problem with respect to the prevention of lower nephron nephrosis. Proper hydration characterized by a normal volume and tonicity of the body fluids is just as important in the protection of the renal tubular epithelium during the administration of sulfonamides, other chemo-therapeutic agents, or transfusions of blood in medical diseases.

One is frequently presented with the clinical problem of a patient who has been oliguric for a matter of hours, arising out of a situation that *may* have produced a lower nephron nephrosis. Overemphasis and overstatement of the dangers of expansion of the volume of the body fluids have engendered a fear of salt and water. In such a situation the assumption is too frequently made that there is, in fact, a state of lower nephron nephrosis. Fluid and electrolyte therapy are now directed toward the prevention of edema; little thought may be given to the problem of the precise pathogenesis of the oliguria. This reduced flow of urine could result merely from an inadequate replacement of fluid losses. The successful repair of such a situation must achieve a restoration of the volume, tonicity, and disposition of the body

fluids. A contracted plasma volume, hyponatremia, or both, may, in themselves, be responsible for a small flow of urine. Acute hyponatremia even in association with an expanded volume of body water can have dire consequences, and if this state is not corrected, a true lower nephron nephrosis may develop. The prompt and adequate correction of these abnormalities encourages a return of renal function to normal dimensions or, at least, may minimize the severity and duration of the disorder. If the correction of these abnormalities fails to induce a proper flow of urine, little would appear to have been lost. This correction need not proceed to the point of inducing edema, although the harmful effects of minimal overexpansion of the extracellular compartment seem negligible. There is ample evidence that a reasonable excess of fluid is to be preferred to dehydration.

There is less controversy[3, 25, 29, 41, 42] concerning the management of the patient with an established lower nephron nephrosis. This is a self-terminative illness, and the aims of therapy are to restore and to maintain the internal environment in a state as near normal as possible, to retard the rate of protein catabolism, and to make it possible for the patient to exploit the earliest return of renal function. Fluids are initially administered in such volume and composition as to compensate for antecedent losses. The tonicity of the body fluids should be brought to and maintained at a normal level. Hyponatremia should be corrected with hypertonic saline. The amount of sodium necessary to raise the concentration of this ion to a normal value must be calculated on the basis of the fact that its osmotic effect is distributed throughout the entire volume of body water, although sodium itself is largely confined to the extracellular compartment.[27, 28] A reduction in the concentration of bicarbonate in the serum in this disorder most commonly results from its displacement by stronger acids. If the concentration of sodium in the serum is normal, this acidosis cannot be modified unless the concentration of sodium is raised above normal. This would substitute the discomfort of thirst for what is usually a symptomless acidosis. However, if hyponatremia and a depressed concentration of bicarbonate in the serum co-exist, some of the sodium may be administered as sodium bicarbonate which will modify the acidosis. Needless to say, peripheral vascular collapse should be treated with whole blood, washed red cells, or a colloidal solution such as albumin, depending on the particular circumstances in a given patient.

Having thus restored circulatory efficiency, and the volume and sodium concentration of the body fluids, only current losses should be replaced. The patient should receive enough water and salt to replace obvious extrarenal losses and enough water, in addition, to provide for losses through the skin and respiratory passages. The latter seldom exceed 1000 cc. a day unless the body temperature is elevated or sweating is profuse. One hundred grams of carbohydrate in doses spread throughout the twenty-four hours

will provide a maximum protein-sparing effect and will retard the rate of accumulation of nitrogenous end-products and potassium. Cardiac decompensation should be promptly treated with a digitalis preparation.

One feature of the treatment during this expectant stage has been much neglected. Absolutely nothing should be given by mouth until the patient has been free from vomiting and nausea for at least twenty-four hours. If this injunction is meticulously observed, the use of tubes and suction is unnecessary. Attention to this detail will minimize extrarenal losses of fluid and electrolytes. No food other than carbohydrate should be given until urination is resumed.

Under such a regimen very few patients will succumb to the effects of the renal insufficiency per se. Most patients can be maintained in a reasonable state of comfort and safety during the time that it may take for the lesion to heal. Stock[40] and others report the survival of patients in a comfortable state under such a regimen for as long as forty-nine days with complete renal insufficiency from neoplastic obstruction of the ureters. A patient (B76973) suffering with both lower nephron nephrosis and carcinomatous obstruction of both ureters, treated by this Service, survived thirty-five days with virtually complete anuria. During the first six days a total of 325 cc. of urine was obtained from the bladder; there was complete anuria during the last twenty-nine days. She was, however, in a state of reasonable comfort until the last days of her illness. There were periods of mental confusion during the last week, and she was almost comatose the last three days. Six days prior to death, chemical studies revealed an NPN of 220 mgm. per cent, serum bicarbonate and chloride of 17.8 and 78.2 mEq./L. respectively, sodium of 131.2 mEq./L., and a concentration of potassium in the serum of only 3.6 mEq./L.

An occasional patient may develop a high concentration of potassium in the serum, which may reach cardio-toxic levels. Elkinton and his co-workers[8] have demonstrated that this ion can be successfully removed with the use of a carboxylic ammonia exchange resin. This may be administered orally or instilled per rectum. This latter route is especially desirable in those instances where nausea or vomiting complicate the clinical picture. They advocate its use twice a day as a ten per cent suspension in a volume of 250 cc. of water. With this technique they were able to remove from twenty-one to seventy-three mEq. of potassium in a day. The concentration of potassium in the serum fell from as high as 7.7 to 5.4 mEq./L. This should be practised, however, only if the concentration of potassium in the serum is known to be high. Moreover, a high concentration of potassium in the serum should raise the question as to whether the administration of fluids and carbohydrate has been well regulated.

The value and proper rôle of the variety of artificial dialyzing procedures remain subjects for investigation.[7, 8, 23] Peritoneal lavage carries a serious

hazard of infection, and intestinal dialysis has been inefficient. It has been convincingly demonstrated by Merrill and his associates[28] and others that an artificial kidney of the Kolff design in competent hands is an efficient and reasonably safe dialyser. It is not certain, however, that the use of this instrument has materially altered the ultimate fate of a patient ill with lower nephron nephrosis. Our service has had no experience with the artificial kidney, and the fatalities with lower nephron nephrosis that have been encountered seem adequately explained on the basis of other aspects of the total disease picture than the renal insufficiency per se. It appears unlikely that the artificial kidney would have prevented these deaths. Further experience with this technique, nevertheless, may establish its utility and limitations in the management of this condition. The use of dialyzing procedures must be accompanied by expert knowledge and accurate chemical control. If these are not available, the use of an artificial kidney may well increase the mortality rate in lower nephron nephrosis.

The patient who is treated along the generally accepted principles of fluid therapy outlined above is in a situation to exploit the earliest phase of recovery. It is most important not to lag behind the diuresis in the prescription of fluids and electrolytes. Every advantage should be taken of the returning renal function to perpetuate and augment the increasing flow of urine. In the early stages of recovery the urine is dilute and its composition reflects a disability on the part of the tubules to differentiate in the reabsorption of water and solutes. This deficiency in tubular function is expressed in a concentration of NPN in the urine which is not much higher than that in the blood, while, since tubular reabsorption of electrolytes is impaired, sodium chloride may appear in relatively high concentration. Therefore, although the urine may contain very little non-protein nitrogen, losses of water and electrolytes may be quite large. If these losses are not replaced, the volume of the urine may be reduced, recovery delayed, and the concentration of NPN in the blood will continue to rise. Within a few days all aspects of renal function begin to improve, and in a few months even the most subtle analyses of renal function will fail to reveal any evidences of this severe disorder.

An attempt has been made in this discussion to focus attention on the *preventive* aspects of treating this disorder. The appropriate administration of fluids under circumstances where lower nephron nephrosis is a threat may prevent the disorder or, at least, reduce it to less disastrous proportions. A careful analysis, with the aid of appropriate laboratory examinations, of the sequence of events leading to a state of oliguria, and correction of the abnormalities of volume and tonicity of the body fluids, may be followed by a gratifying diuresis. A process that might have become lower nephron nephrosis may be forestalled. The maintenance of an internal environment as nearly normal as possible, by the intelligent administration

of water salts, and carbohydrate, will sustain a patient during the period of complete renal insufficiency and place that patient in an optimal state to respond appropriately when the lesion is repaired.

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PRELIMINARY OBSERVATIONS ON BLOOD COAGULATION UTILIZING ULTRASONICS FOR CONTINUOUS MEASUREMENT OF VISCOSITY*

RAYMOND YESNER, ALFRED HURWITZ, STANLEY R. RICH, WILFRED ROTH, AND MARTIN E. GORDON

A new technique for continuous measurement of viscosity† and its application to studies of blood viscosity are described in this communication. For more than forty years viscosimetric studies of blood have been based on static, discrete determinations, such as that of Hess[1] in which viscosity was considered to be inversely proportional to volume under given conditions of pressure in capillary tubes. The closest approach to continuous measurement of viscosity was described recently by Kristenson,[2] who attached a small pendulum to a container of blood and recorded the amplitude of the swings. By a large series of recordings, a "coagulation profile" of the blood was constructed.

The viscosity of materials that change their physical characteristics with time, such as blood during clotting, is difficult to determine unless it can be measured instantaneously, continuously, and automatically. In August, 1950, a joint research program was undertaken to adapt a new ultrasonic instrument‡ to dynamic studies of blood viscosity during clotting.

The Ultra-Viscoson is an electronic instrument which uses ultrasonic waves to measure the viscosity of Newtonian liquids and the apparent viscosity of non-Newtonian liquids continuously and automatically. It comprises an ultrasonic probe and an electronic automatic computer. The sensing element of the probe is a thin blade of steel alloy which protrudes from a stainless steel barrel (Fig. 1). Ultrasonic waves are produced by the probe in the liquid in which it is immersed. The electronic computer, attached to the probe by a cable, instantly calculates the viscosity of the liquid from the effect of interaction of the ultrasonic waves with the molecules of the liquid. If sufficient liquid surrounds the probe, minute layers are caused to slip back and forth over one another at an ultrasonic frequency, with bi-directional or alternating motion. The electronic computer instantaneously computes the force required to produce this sliding motion, and this force is proportional to the viscosity of the liquid.

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† Viscosity is that property of a fluid that resists flow. If two parallel plates each one cm.^{2} in area are separated by a layer of liquid 1 cm. thick, the viscosity of this liquid, by definition, will be 100 centipoises if one dyne of force is required to pull one plate parallel to itself at a velocity of 1 cm./sec. with respect to the other plate.

‡ The Ultra-Viscoson is manufactured by the Rich-Roth Laboratories of East Hartford, Conn.

Received for publication December 4, 1951.

The operating frequency of the Ultra-Viscoson is 28 kilocycles per second. The peak amplitude of vibration of the probe is less than one micron; the ultrasonic energy in the liquid is in the order of microwatts. This level is deliberately designed to be very low—far below cavitation levels—so as to eliminate any effects that the ultrasonic energy will have on the characteristics of the test sample. This fact was established for blood by switching the instrument on and off during the clotting process and observing that the progress, slopes, and magnitudes of the curve were unchanged. When the instrument was switched on after an "off" period, the recorder pen returned to that position on the curve which it would have reached had no interruption occurred.

The Ultra-Viscoson measures the absolute viscosity of Newtonian liquids from 0 to 50 centipoise $\times$ gm./cc. in 4 ranges: 0-50, 0-500, 0-5,000, and 0-50,000. The instrument reproducibility is $\pm$ 2% for liquids having reproducible rheological properties, whether Newtonian or non-Newtonian. The absolute accuracy for Newtonian liquids in the 0-50 centipoise gm./cc. scale (which is the scale used in these tests) is $\pm$ 2%.

Once a sample depth of 0.3 cm. is exceeded, the instrument reading is given by

$$\text{Meter reading} = \frac{\rho}{\omega_0}\left(\sqrt{g^2 + \beta^2\eta^2} + g\right)$$

Where ρ = density of liquid — gm/cc.

ω_0 = constant of the system

g = rigidity coefficient of liquid — $\frac{\text{dynes}}{\text{cm.}^2}$

η = viscosity coefficient of liquid — poises

β = frequency employed

For the majority of liquids, including blood, the rigidity is completely negligible and $\beta = \omega_0$. Therefore, meter reading $= \rho\eta$ is the response of the instrument obtained. In this range the effect of frequency change is negligible.

In operation, a standard recorder such as the Brown Electronik is attached to the Ultra-Viscoson to obtain a permanent graph. The probe is mounted in a siliconized glass tube, with which it must not come in contact, and partially submerged in a water bath at 37° C. Only 4 cc. of blood are necessary to cover the probe. The recorder is started the moment blood appears in the siliconized venipuncture syringe and before its transfer. The instrument measures 0 at this point, since the blade is in air.

A total of 175 graphs have been drawn and analyzed in patients with various medical and surgical condition and in normal, healthy adults. The latter group comprises 57 males and 10 females, on whom replicated data were obtained in six males and four females. The replicated data were averaged in each case, so that a total of 67 graphs have been analyzed as a basis for this study. Each chart presents an initial rise, a drop back to a lower plateau, and a second main rise which levels off after a variable time, and in some instances drops again. A mean has been established for six points on the chart from 67 graphs drawn from the normal controls and a mean chart has been constructed (Fig. 2). "A" is the peak of the initial rise and has a mean height of 21. The probable error of the mean is calculated to be 0.5; the probable error of a single observation, 3.0. The ordinate scale is labeled, K $\times$ $\sqrt{\text{centipoises} \times \text{gm./cc.}}$ When the instrument is calibrated vs. a Newtonian viscosity standard oil, K $= \sqrt{200}$. Scales are being printed in centipoises $\times$ gm./cc. units, so that the conversion factor will be eliminated and the recorder as well as the computer indicating meter

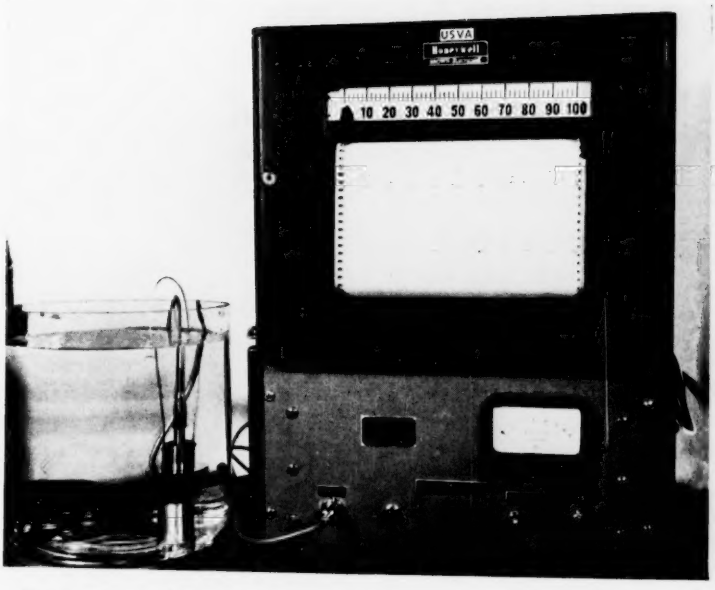

FIG. 1. Photograph of the entire apparatus including the water-bath which contains the immersed glass tube and probe, and the Ultra-Viscoson with the Brown Electronik recorder on top.

will be direct reading. "B" is the point on the plateau at which the main rise begins. It has a mean height of 15. The probable error of this mean is calculated to be 0.3; the probable error of a single observation, 2.3. Furthermore, it occurs at a mean time interval of 3 minutes and 10 seconds from the moment blood is drawn. The probable error of this mean is calculated to be 5 sec.; the probable error of a single observation, 43 sec. No single value for this determination exceeded 5 min. in normal individuals. This point is considered to be one of the highly significant values of the curve. It measures the latent period before steady increase in viscosity begins. The decline from A to B results from the process of attainment of equilibrium by molecular migration to produce a minimum potential energy at the probe to blood interface. This interesting and potentially useful phenomenon is under more thorough investigation. (Patients with a tendency to bleed may show greatly prolonged values for "B," as in a pseudohemophiliac with normal bleeding and clotting times, but with an abnormally prolonged "B" time of 15 minutes [Fig. 3]. After administration of fresh frozen plasma or fresh whole blood, viscosimetric graphs of this patient's blood were of the normal type, as in Figure 2. This case will be reported later in detail.)

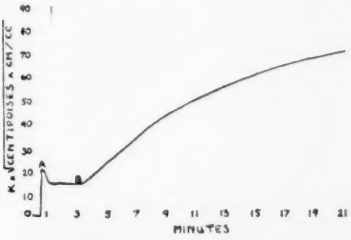

FIG. 2. The mean curve constructed from 67 normal curves. A = apex of initial rise with mean height of 21. B = starting point of second or main rise with mean height of 15 and mean time interval of 3 minutes 10 seconds from venipuncture. Level 20 on the graph is reached at a mean time interval from B of 1 min. 40 sec.; level 30, at a mean time interval from 20 of 1 min. 50 sec.; level 40, at a mean time interval from 30 of 2 min. 10 sec.; level 50, at a mean time interval from 40 of 3 min. It is unusual for this point to be more than 12 min. from 0.

Level 20 on the main rise has a mean value of 1 min. 40 sec. from "B," Level 30 has a mean value of 1 min. 50 sec. from 20. Level 40 has a mean value of 2 min. 10 sec. from 30. Level 50 has a mean value of 3 min. from 40. The upper reaches of the curves are approximate because the computer was not standardized as carefully in this area in the early experiments as more recently. Since most of the curves flatten out at variable levels no further means were established. A small number of curves show a slight decrease in viscosity in this area. The points enumerated demonstrate the incline of the slope, which present experiments indicate may be important in differentiating certain pathologic states.

Flattening out of the curve below 50 is unusual and probably abnormal. In one patient on dicumarol therapy with a prothrombin time of 10% and spontaneous bleeding, a value of only 33.5 was reached in 53 minutes. In another patient who showed a marked bleeding tendency following a major operation, a level of 44 was reached in 14 min. 35 sec. (normal mean,

9′ 25″), falling off to 38 in 29 min. 20 sec. (Fig. 4). After intravenous injection of calcium gluconate, a normal viscosimetric curve was obtained in this patient.

In most cases, Lee-White clotting times were run simultaneously with the viscosimetric measurement. A firm coagulum usually occurred after "B" along the main rise, without any indication on the graph. It is beyond the scope of this preliminary paper to answer many questions concerning what is being measured by the instrument during the process of whole blood coagulation. On a sample of whole blood, taken from a normal control, when the computer was well calibrated, point A was 19.7 and point B, 17. Oxalated blood plasma taken from the same blood sample gave a flat reading of 16. Blood serum from the same sample gave a flat reading of 13.3. The serum level of 13.3 is calculated to be 1.1 centipoises. (Glasser[3] gives 0.9-1.2 centipoises as normal serum range). The plasma level of 16 may be calculated to be 1.3 centipoises (Glasser gives 1.0-1.4 centipoises as normal plasma range). It is interesting that the A level of 19.7, which is calculated to be 2.0 centipoises, is less than the standard viscosity range of whole blood (Glasser gives 2.5-3.5 centipoises as normal blood range). The B level of 17 or 1.5 centipoises, is greater than that of plasma from the same sample.

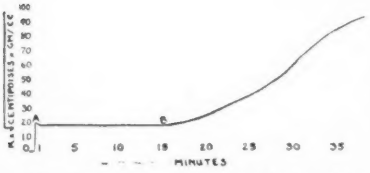

FIG. 3. The curve of a pseudohemophiliac during bleeding episode showing a prolonged plateau before the main rise. B lies at 15 min. from 0. The early part of the main rise is long and flat, level 50 occurring at 27 min. 35 sec. from 0. The latter part of the main rise is unusually steep.

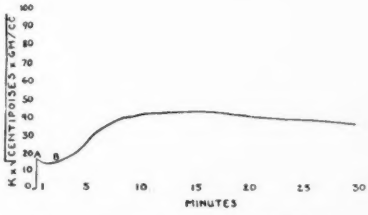

FIG. 4. The curve of a patient with gastrointestinal bleeding and hypocalcemia, showing the apex of the main curve at 44, with a subsequent drop to 38.

No significant sex difference is apparent with the data on hand. Viscosity curves on women are being collected at different points in the menstrual cycle and during pregnancy and will be reported when more data are available.

Reproducibility is excellent. The following values were obtained from a female donor at 1:25 P.M. and at 3:00 P.M. on November 26, 1951 (last menstrual period, November 8):

	A	B
1:25 P.M.	20	15.5, at 3 min. 10 sec.
3:00 P.M.	20.5	16 , at 3 min. 10 sec.

Summary

A new technique for continuous measurement of viscosity of blood during coagulation has been presented with an analysis of viscosity curves from 67 normal, healthy adults. Two abnormal curves from "bleeders" are shown. Viscosimetric studies are now being carried out in relationship to phlebothrombosis, myocardial infarction, dicumarol therapy, carcinomatosis, pregnancy, in pre- and postoperative periods, and in patients with abnormal bleeding tendencies, as well as in further investigation of normal blood.

Acknowledgment

We wish to express our indebtedness to Mr. Arthur Rauch for technical assistance during these experiments.

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EVENTS

YALE MEDICAL SOCIETY

November 14, 1951

THE BIOSYNTHESIS OF FATTY ACIDS AND CHOLESTEROL AND THE HORMONAL CONTROL OF THESE PROCESSES. By Samuel Gurin, Professor of Physiological Chemistry, University of Pennsylvania.

The influence of hormones upon synthesis of long-chain fatty acids from radiocarbon-labelled acetate by rat and cat liver slices was described. Insulin increased the synthesis of fatty acids, but it did not reverse the diminished synthesis demonstrated by liver slices of pancreatectomized or alloxan-treated animals. Liver slices of hypophysectomized animals synthesized fatty acids to a greater extent than livers of intact animals. Extirpation of both the pancreas and the hypophysis did not influence the amount of fatty acids formed, but the addition of purified growth hormone to the liver slice of such an animal lowered the fatty acid production. Growth hormone itself was without effect on the yield of fatty acids, although it antagonized the increased synthesis engendered by insulin. There was no quantitative difference between the production of fatty acids from acetate by normal livers and production by livers of pancreatectomized and adrenalectomized cats. Addition of cortisone lowered fat synthesis.

Production of fatty acids from acetate by pigeon liver homogenates was markedly increased by forced feeding of glucose to the pigeons; for maximum synthesis of fatty acids from such homogenates, both particulate and supernatant portions of the homogenate were required. The essential components of the supernatant appeared to be ATP and magnesium.

It is possible that in diabetic states, conversion of two carbon fragments to fatty acids is blocked, and an alternative metabolic pathway is taken, leading to the formation of excess ketone bodies and cholesterol.

J. P. G.

PHARMACOLOGY SEMINAR

November 15, 1951

SOME METABOLIC FUNCTIONS OF FOLIC ACID AND VITAMIN B_{12}. By Frank H. Bethell, Professor, Department of Medicine, University of Michigan, Ann Arbor, Michigan.

Folic acid occurs as a conjugated molecule in most natural food stuffs; it is split by the gastrointestinal juices and absorbed as free folic acid. Pernicious anemia patients show a marked reduction in the renal excretion of free folic acid as compared with normal individuals. After several days of oral administration of free folic acid to these patients, their folic acid excretion approaches normal values. If, however, conjugated rather than free folic acid is given, they show no increase in folic acid excretion. Conjugated folic acid previously treated with enzymes obtained from the intestinal mucosa of hogs gives a response similar to that of free folic acid. The effects of free and conjugated folic acid on reticulocyte response parallel the effects on renal excretion.

Investigation suggests that additional B_{12} synthesis occurs in the large intestine, but that this is lost to the flora of the small intestine which need B_{12} for growth. Pernicious anemia patients given aureomycin showed a good reticulocyte response in about three months; when the antibiotic was

supplemented with B_{12}, the response was more rapid. B_{12} given alone (i.e., unprotected) would not elicit a reticulocyte response; but when it was given with dialyzed extract from hogs' duodenal mucosae, a good response was obtained though the latter preparation was also ineffective when given alone.

These observations suggest that the "extrinsic factor" is B_{12}, as is the anti-pernicious anemia factor of the liver, and that the "intrinsic factor" is something that enables the B_{12} to get through the intestinal barrier.

K. G. B.

BEAUMONT CLUB

November 16, 1951

COMMENTS ON CHARLES DARWIN. By Paul B. Sears, Professor of Conservation, Yale University.

Charles Darwin grew up in a culture ripe for change; he left the world much different than he found it. His father sent him to Edinburgh to study medicine but he soon gave up the idea—operations without the benefit of anesthesia especially disturbed him. Darwin then enrolled at Cambridge to take holy orders, but found that none of his classes appealed to him as much as his extracurricular activities in natural history. The time drew near for his ordination but, fortunately for him, a British naval vessel was ready to go to South America for a cruise and it was arranged that Darwin should go along as naturalist. The five-year voyage was an unusual experience; the South American continent had never before been surveyed by a naturalist. The pattern of organisms and their adaptations soon became his special interest. Mutability was an idea centuries old, and on this voyage Darwin was able to picture it and set down the organization. He had never had any grave doubts about religion, although he questioned parts of the creed of the Church of England. But on the South American trip any remaining belief in the "literal truth" of the Scriptures dropped "like a cloak" from him.

By implication Darwin laid the foundation for two new sciences: (i) Genetics—an explanation for changes and variations in organisms, and (ii) Ecology—the relationship of organisms to their environment. In the twentieth century each has come into its own.

J. K. R.

NEUROLOGICAL STUDY UNIT

December 3, 1951

THE EFFECT ON PERSONALITY OF DIFFERENT INCISIONS IN THE FRONTAL LOBE. By Dr. Asenath Petrie, London, England.

Seventy individuals, previously psychologically tested, submitted to lobotomy and were followed for a minimum period of three months. The tests were designed to indicate the degree of change in "neuroticism," intraversion, and intelligence. A surgeon performed in the various subjects two different types of lobotomy—one a posterior cut (Brodmann areas 9, 10, 11, 47, 45), the other an anterior cut (Brodmann areas 9, 10, 11).

Temperament and character following lobotomy appeared to be the same regardless of the type of cut. Investigators noted that patients blamed themselves less, preoccupied themselves with the present rather than the past, found that time passed much faster. Tests indicated a decided misuse of

words in conversation and some slow but accurate efforts in unscrambling proverbs. Their sense of humor, particularly sex humor, was better.

Changes seemed to be more pronounced following the posterior type operation. The anterior operation appeared to avoid any loss of I.Q. as determined by the Wexler test. Tests following either type of lobotomy cut clearly showed less "neuroticism" and introversion in patients. There was a hint of left dominance in right-handed individuals following some of the procedures.

J. K. R.

YALE MEDICAL SOCIETY

December 12, 1951

THE CUMULATIVE EFFECT OF THE PARENTAL AGING PROCESS ON CHEMICALLY INDUCED FIBROSARCOMA IN THE OFFSPRING. By Leonell C. Strong.

Methylcholanthrene-induced fibrosarcoma was studied in successive litters of tumor bearing mice. A sex differential was noted with the males, but not the females, showing no decreased survival time in later litters. The period of latency of the tumor, which changed little in the females, started out being longer in the males, but by the 14th generation reached the same length as that of the females. The incidence of tumors increased with successive litters in both sexes.

Apparently the offspring do not directly duplicate parental norms, but rather reflect the biological state of the parents at the time of conception. Susceptibility to tumors has especially been demonstrated to increase with the ageing process of the parents, with the parental age reflecting ageing of the species itself. Finally the current rise in human cancer was tentatively correlated with the decreasing size of the family and the increasing maternal age.

N. A. H.

USE OF THE CUVETTE OXIMETER DURING CARDIAC CATHETERIZATION. By H. S. Harned, Jr., Paul R. Lurie, and Ruth Whittemore.

An evaluation was made of the accuracy of the cuvette, or whole blood, oximeter in measuring absolute oxygen saturations, and of the usefulness of the oximeter as an aid to the operator during cardiac catheterization.

Catheterization studies on forty children were conducted and one hundred ninety determinations were obtained for comparison with gasometric analyses of blood samples. The standard deviation of the difference between the two sets of determinations was 4.5% for all the range of values and 3.56% for gasometric saturations above 70%. The error in this range suggests that the cuvette oximeter values would not be adequate substitutes for gasometric analyses under the conditions of this study. However, the cuvette oximeter promises to have several valuable uses: first, as a quick method for locating the catheter tip for representative sampling; second, as an aid to presumptive diagnosis during cardiac catheterization so that the procedure might be adapted to the individual patient's problem; third, as a means of identifying an unusual site of the catheter tip; and fourth, as a sensitive means of detecting cardiac embarrassment during the procedure. The cuvette oximeter measures differences in oxygen saturation more accurately than it measures absolute values.

J. F. S.

THE INFLUENCE OF THE TONICITY OF BODY FLUIDS ON THE RATE OF EXCRETION OF ELECTROLYTES. By J. H. Darragh, L. G. Welt, A. V. N. Goodyer, and W. A. Abele.

The influence of the tonicity of the body fluids on the rates of excretion of electrolytes was examined in four healthy young adult male subjects. Infusions of isotonic solutions of sodium chloride were administered after a control period during which the tonicity of the body fluids was either (1) not altered, or (2) reduced by achieving a positive balance of water of about two liters. This latter was accomplished with an infusion of 5% glucose.

The rates of excretion of sodium and potassium were lower during the dilution studies both before and after the administration of the saline. In contrast, the rate of excretion of ammonia was higher both before and after the infusion of saline in the dilution studies.

The rate of excretion of sodium is influenced, presumably, by the decrease in the effective osmotic pressure of the extracellular fluid or its necessary consequence—an increase in cellular hydration. Since cellular dehydration is alleged to augment the excretion of potassium, it is likely that the overhydration of the cells is responsible for the diminished rate of excretion in these studies with dilution. The increase in the rate of excretion of ammonia may be the consequence of an enhanced reabsorption of sodium under circumstances of a decrease in the rate of excretion of potassium.

J. H. DARRAGH

THE BRONCHOPULMONARY VENOUS SYSTEM IN DISEASE. By A. A. Liebow.

It is now a well-established fact that anastomoses between pulmonary and systemic trees exist and may enlarge to functional significance in pulmonary disease. Studies of injection casts of the bronchial and pulmonary venous trees reveal that anastomoses exist as well between these systems. The anastomoses and bronchial veins themselves are demonstrably enlarged in casts made of the lungs of patients with bronchiectasis or congenital heart disease. Whereas in the normal lung the bronchial veins are not demonstrated by injection beyond the third order bronchi, they may be injected as far as the 7th or 8th bronchi in a bronchiectatic lung.

There is no method of determining the magnitude of the shunts involved, or indeed of determining the direction of flow in these shunts. It may vary. The direction of the valve at the junction of the bronchial and azygos vein is such as to prevent right to left shunt in the normal lung, but dilatation of the vein in disease may make this valve incompetent. This is suggested by the possibility of retrograde injection of the bronchials from the azygos in diseased specimens. The occasional occurrence of cyanosis following digitalization of patients with cor pulmonale may depend on pressure changes in the auricles such as to cause such a right to left shunt.

W. P. McN.

BOOK REVIEWS

STATISTICS FOR MEDICAL STUDENTS. By Frederick J. Moore, Frank B. Kramer, and Robert G. Knowles. Philadelphia, The Blakiston Company, 1951. viii + 113 pp.

The widespread need for more and improved techniques among medical investigators scarcely needs mentioning. This small book offers the essentials of commonly used statistical methods with a minimum of verbiage. The emphasis is placed on understanding the logical basis rather than the mere setting down of formulae and tests. The development is straightforward and economical; it is more rigorous than some elementary texts for biologists, but no effort is made to demonstrate the fundamental derivations.

Most of the work deals with normal distributions, criteria for normalcy, and tests of significance. One chapter deals with non-normal distributions, and there is a short discussion of design of experiments and presentation of results.

The book is valuable for one who wants some insight into the underlying philosophy of statistics, its possibilities and limitations. The investigator who wants ready-made formulae and tables for rote use will find this presentation too general.

W. P. McN.

RENAL PELVIS AND URETER. By Peter A. Narath. New York, Grune and Stratton, 1951. xii + 429 pp. $12.50.

This book begins appropriately with the embryological development of the ureter and renal pelvis, followed by a discussion of anomalous and malformative accidents that may occur in the upper urinary tract. The thoroughness with which this part of the discussion is developed may be inferred from the fact that it comprises nearly one third of the entire book. Next is described the normal anatomy and histology of this region. The second half of the volume contains a discussion of such intriguing subjects as dynamics and tonus of the upper urinary tract and resorption and absorption from the renal pelvis and ureter. There follows a fine treatment of these physiological problems covering both theoretical and experimental material. The closing chapters describe the historical development and modern technique of diagnostic procedures used to explore the upper urinary tract, including a comparison between retrograde and intravenous urography.

The author has put into this book much that is original with him. Some of the descriptive terminology is new, but logical, and therefore easily adapted to. A unique contribution is made by this volume through its remarkable illustrations and x-ray reproductions. Beside each roentgenograph there is a miniature outline of the renal pelvis sketched clearly in black and white so that no point is lost through inexperience in reading x-rays. The text has been lucidly written and well edited.

V. S. G.

ALGAE OF THE WESTERN GREAT LAKES AREA. By G. W. Prescott. Bloomfield Hills, Michigan, Cranbrook Institute of Science, 1951. 946 pp. $10.50.

This book describes and illustrates all of the known fresh water algae of Michigan and Wisconsin except for the diatoms (a class of yellow-green

algae) and the desmids (an order of green algae) which are such large groups that they merit special treatment.

The plan followed is that usual for handbooks of this sort. Following introductory sections on geographical and ecological principles and observations, there is a descriptive account of the 1100-odd species involved, arranged essentially according to the classification of G. M. Smith. The excellent illustrations, made from zinc plates, are collected into one section, followed by a particularly large bibliography. References are given at division and class headings to the important papers dealing with the morphology, taxonomy, and reproduction of the algae concerned. This is rather helpful to anyone working intensively with one group.

The identification of algae is not a particularly simple task at best. Because the descriptions in this book are clear, because it follows new taxonomic ideas, and because the semidiagrammatic illustrations stress the distinguishing features between species, this book should be very helpful to those who must identify algae, especially if they must go as far as the species. One hopes the desmids and diatoms will receive as good treatment in the near future.

It should be mentioned that the algae of the Great Lakes area are members of widespread groups, which means that the usefulness of the book is not limited to this region.

R. R. L. G.

PHYSICAL BIOCHEMISTRY. By Henry B. Bull. 2d ed. New York, John Wiley and Sons, Inc., 1951. 344 pp. $5.75.

The new edition of Dr. Bull's textbook gives evidence of the progress that has been made in physical biochemistry since 1943 when this book first appeared. Recent advances have been accommodated to some extent by reorganizing the text to obviate repetition. Thus, the chapter, "Membranes and Cell Penetration," has been eliminated, but the information has been retained and interwoven into the discussions of electromotive force cells, ions in solution, and diffusion. Similarly, there is no longer a separate heading, "Electrostatics and Dielectrics"; these subjects are described for the most part in the chapter, "Atoms and Molecules." The integrative nature of the text has been enhanced by placing both the chapter on osmotic pressure and that on electrical conductance in a more logical sequence.

This revised edition is of even greater value than its predecessor, especially to graduate students in the biological sciences eager to gain an acquaintanceship with the applicability of physical chemistry to the elucidation and interpretation of biological phenomena. An expansive bibliography including references to the 1950 literature is available for those interested in special topics.

Lengthening of the pages by three-quarters of an inch has provided larger margins, which, combined with a handsome binding, make it an attractive volume.

J. P. G.

A SYLLABUS OF LABORATORY EXAMINATIONS IN CLINICAL DIAGNOSIS. Thomas Hale Ham, Ed. Cambridge, Mass., Harvard University Press, 1950. 496 pp. $5.00.

COLOR ATLAS OF MORPHOLOGIC HEMATOLOGY. Geneva A. Daland. Thomas Hale Ham, Ed. Illustrated by Etta Piotti. Cambridge, Mass., Harvard University Press, 1951. 74 pp. $5.00.

With the collaboration of thirty-two contributors Dr. Ham, formerly of the Harvard University School of Medicine and now at Western Reserve University School of Medicine, has gathered a tremendous number of laboratory examinations into a single volume, the *Syllabus,* intended for use by medical students (to whom it is dedicated), laboratory technicians, and practising physicians. A fine introductory unit discusses such miscellaneous topics as planning the diagnostic procedure, limits of accuracy of diagnostic tests, care of equipment, costs, and normal values. Thereafter, a separate unit is devoted to diagnostic examinations which pertain to each of the following entities: blood, urinary system, gastrointestinal system, infectious diseases, diseases of metabolism, and extravascular fluids. In each unit, following an introductory paragraph, each test is described in relation to its "clinical value, availability, cost, principle, method, and limitations on interpretations of the test itself and as a diagnostic aid related to the history and physical examination of the patient."

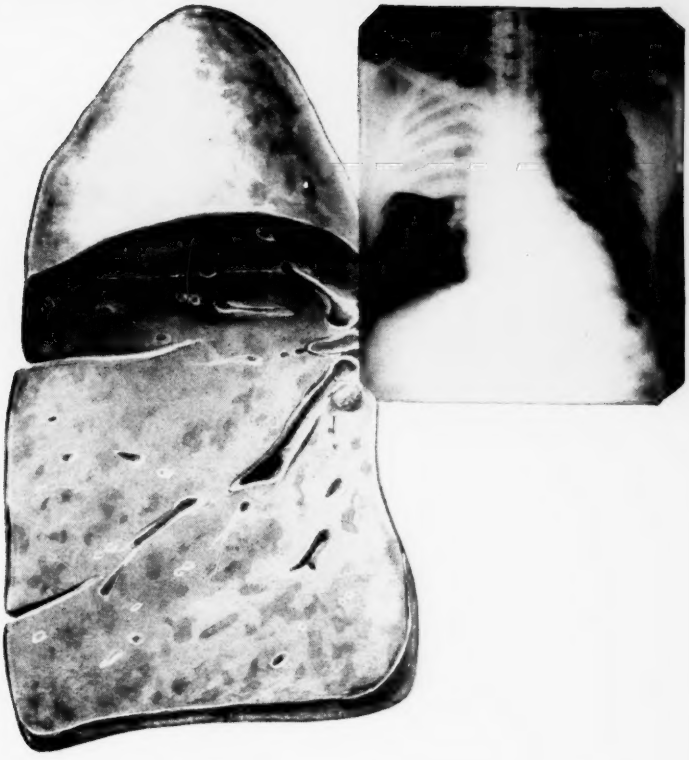
Practicability and simplicity are given high priority. The limits of accuracy of each test are emphasized. Technique is described clearly, and pitfalls are indicated. The authors do not attempt to present differential diagnosis. They describe each procedure per se and the objective findings which can be elicited, leaving the interpretation and application to the judgment of the reader.

This is a tremendous volume. It measures 8½" by 11", 496 pages, and is paper bound. The composition is by Varityper, from which the format suffers in the charts and tables; but the price of the *Syllabus* is thus kept low. The bibliography is voluminous and the index is adequate. The literary style throughout is extraordinarily brief and clear, qualities which are particularly desirable in describing technical procedures. When one reads about a procedure in this book, one feels that he has not only learned the technique, but appreciates as well the meaning and significance of the procedure.

The companion volume to the *Syllabus* is the *Color atlas.* In fourteen colored plates and eight black and white diagrams it presents the normal and pathological morphology of the peripheral blood elements under Wright's stain. The material is directed toward the diagnosis and treatment of blood disorders rather than toward theories of blood cell development. The text describes and interprets the morphology seen in the plates. In each condition a case history is presented, the blood picture seen in the color plate is described, and the interpretation is offered.

The *Color atlas* is bound in a stiff cover. An index and a bibliography of 193 entries are appended. In keeping with the spirit of the *Syllabus* the author and editor have weighted the presentation in the *Atlas* toward diagnosis instead of theoretical considerations. The colored plates are outstandingly clear. The blood elements as pictured are very readily recognized in actual smears, and the *Color atlas* should be a very real help to the student and to the unexperienced hematologist. The only regrettable fact is that there is no colored plate of the developmental order of the white cell series. The combination of *Syllabus* and *Color atlas* deserves the highest recommendation.

J. F. S.



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Butterfield, J. G., and Starkweather, S. A.: J. Philadelphia General Hosp. 4 5, Jan., 1951.

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